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AMERICAN MALACOLOGICAL BULLETIN

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Diversity and durability: responses of the Madeiran and Porto-Santan snail faunas to natural and human-induced environmental change

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Abstract: The snail fauna of the Madeiran Archipelago has the high regional diversity and endemism, and high species and genus differentiation between islands characteristic of oceanic island faunas. It is unusual in two respects: the existence of good Pleistocene/Holocene fossil records, and the apparent durability of the endemic fauna in the face of massive human disturbance and the introduction of non-indigenous species.

We use the fossil record, the present microdistributions of species, and the environmental records available to track the generation of diversity and the response to human disturbance. The nature of the environment available and the life-habits of endemic species appear to account for the relative resilience of these species.

Land snail faunas of volcanic oceanic islands are usually characterized by very high levels of species diversity and endemism (Solem, 1983, 1984, 1990a). Sample site diversities are not high; there is marked microgeographical differentiation within islands, and evolution at species level occurs mostly within rather than between islands. Overall species diversity in archipelagos is thus often very high indeed (Solem, 1990b).

This situation offers excellent opportunities to study the process of speciation (Clarke and Murray, 1969; Cowie, 1992a) but these are often compromised by the extreme vulnerability of such faunas to habitat destruction and the introduction of predators and competitors (Cowie, 1992b). Thus three-quarters of the endemic fauna of the Hawaiian archipelago is believed to be extinct (Hadfield, 1986; Solem, 1990a, b) and a similar situation occurs on other Pacific islands. Extinction is often very recent: Tomiyama and Kurozumi's (1992) studies on the Ogasawara Islands south of Japan indicate that nearly 40% of endemic species have become extinct since 1945, with higher figures on the most disturbed islands.

Not all oceanic islands have been so drastically affected; the islands of the Gulf of Guinea, retaining some primary forest and lacking introduced predators retain most if not all of their described endemic faunas (Gascoigne, 1994).

While similar in most of these respects to the faunas of other oceanic islands, that of the Madeiran Archipelago differs in that, despite over 500 years of human settlement and disturbance, it has apparently suffered much less. In this paper, we offer an explanation of this robustness, based on analysis of past environmental changes both natural and human-induced. Subfossil, nineteenth-century, and modern records are available as evidence.

Cameron and Cook (1992) gave a general account of the development of faunal diversity. Environmental interpretation and estimates of extinction are made easier by the existence of snail-rich late Pleistocene and Holocene deposits spanning the last 300,000 years (Cook *et al.*, 1993), and by the application of amino-acid epimerization techniques to the dating of individual shells (Goodfriend, 1987, 1991; Goodfriend *et al.*, 1994).

THE ARCHIPELAGO AND ITS ENVIRONMENTAL HISTORY

Together with the Deserta Islands, Madeira (ca. 740 km^2) and Porto Santo and its neighbouring islets (ca. 41 km^2) constitute an archipelago of volcanic, oceanic origin. Madeira is mountainous, rising to over 1800 m, and contains a wide range of habitats and climatic zones, including subalpine regions, extensive laurel forests, and lower coastal areas with an approximately Mediterranean climate. Although some rocks are 15+ million years (MY) old, there have been more recent periods of volcanic activity, the last perhaps 0.75 MY ago (summary in Mitchell-Thomé, 1985). Substrata are almost exclusively volcanic in origin, and coasts are generally rocky and steeply shelving.

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Only at the eastern extremity of the island are there soils of sea-sand origin on a low peninsula which is the driest part of the island. Ecologically, this peninsula now resembles parts of Porto Santo.

Porto Santo differs in many respects. One hill only rises just above 500 m; rocks are all dated to 12-14 MY old (Mitchell-Thomé, 1985), and with the exception of sea-cliffs to the northeast and some hill summits, the landscape is rounded, with sometimes massive colluvial deposits on hillsides. There is an extensive sandy beach along the southern coast, and sandy deposits cover most of the land below 100 m. They sometimes ascend higher, and the low center of the island is entirely covered by them, separating the rocky hills to the west and east. Rainfall is lower than on Madeira. No natural forest survives, and except for recent forestry plantations, the habitats available are heavily grazed grassland (often with bare, eroding sand) or a very open shrubby environment resembling Mediterranean garrigue or phrygana.

Human occupation of the islands started in 1419-1420 A. D. Apart from widespread habitat destruction, there have been numerous deliberate and accidental introductions of plants and animals. Substantial areas of both islands are now dominated by non-native vegetation. There are no indigenous terrestrial mammals, but goats, sheep, rabbits, mice, and rats have all been introduced. There are many non-endemic mollusks, most of which have probably been introduced (Waldén, 1983; Goodfriend *et al.,* 1994), and these include Zonitidae with carnivorous habits.

Analysis of the stratigraphy and subfossil snails in the sandy deposits of Madeira demonstrates not only the locally catastrophic consequences of human disturbance and introductions (Goodfriend *et al.,* 1994) but also the occurrence of Pleistocene and pre-colonization environmental changes between more wooded (damp) and open (dry) landscapes. These sequences are interrupted by massive accumulations of fossil-free sand, indicating periods of dune mobility and (presumably) localized faunal exterminations (Cook *et al.,* 1993; Goodfriend *et al.*, 1996).

Analyses of the far more extensive deposits on Porto Santo are not yet complete, but it is clear that similar fluctuations occurred there, including drastic changes in lowland areas following human occupation. Unlike Madeira, Porto Santo is surrounded by a shelf of shallow water; sea-level depression in full glacial periods of the Pleistocene would not only have greatly increased the size of the island, but would also expose sand subsequently blown inland. Periods of dune mobility would isolate eastern and western hills (Cameron *et al.,* 1996).

MATERIAL

The land snail fauna of the archipelago has been studied intensively since the 1820s, and Wollaston (1878) gave a remarkable and detailed account of the living and subfossil fauna then known. Many workers have studied the fauna in the second half of the twentieth century; Waldén (1983) provided an annotated checklist for the archipelago, to which later workers have made minor alterations and additions (Groh and Hemmen, 1984, 1986a, b; Hemmen and Groh, 1985; Holyoak and Seddon, 1986; Cook *et al.,* 1990; Seddon, 1990; Rähle, 1992).

In addition to work already published, we have drawn on data from our survey of laurel forests and coastal scrub in northern Madeira, and on a comprehensive survey of Porto Santo, the results of which will be published elsewhere. We thus have data on species diversity and occurrence from subfossil material, from good nineteenth century accounts, and from recent surveys which include detailed distributional data. Similar comprehensive surveys of the relatively inaccessible Deserta Islands have not been carried out, and we have not considered their fauna.

There are inevitable difficulties of taxonomic interpretation involved in such comparisons. We have chosen to ignore subspecific taxa, because early workers, including Wollaston, appear not to distinguish reliably between geographically replacing forms and varieties found at the same site. In general, we have accepted Waldén's (1983) nomenclature and assignments of taxonomic rank, with some adjustments made in the light of later work. Minor changes in assignment would make little difference to our conclusions. A list of species and their status is given in the Appendix.

There are also some difficulties in interpreting nineteenth century statements concerning abundance and distribution. Wollaston (1878), while generally meticulous, occasionally admitted to conflating samples from different localities, and sometimes appears to rely on distant memory. Contemporaries were often far less reliable. Twentieth century records are generally accurately localized.

DISTRIBUTION PATTERNS AND DIVERSITY

On both Madeira and Porto Santo, sample site diversities are modest, being mostly in the range of 5-15 species (Solem, 1984). While there are clear differences in habitat between some species, there is also evidence of geographical differentiation between faunas drawn from the same habitat (Cameron and Cook, 1992; Cook *et al.*, 1993; Cameron *et al.*, 1996).

The range of available habitats on Madeira is considerable. Faunas from laurel forests at mid- to high altitudes (500+ m) show little geographical variation, while those from drier environments at lower altitudes show much more. While not entirely consistent, these differences can be attributed to climatic fluctuations which caused periodic isolation of fragments of dry, open, lowland habitats (Cook, in press).

The range of habitats is much more restricted on Porto Santo, and there are numerous cases of allopatric replacements of sibling species in similar environments (Fig. 1A). These are examples of non-adaptive radiation in the sense defined by Gittenberger (1991). While there are some habitat differences between species, most of the diversity is related to the isolation of hills caused by sand movement between them. The recent human disturbance has created a number of vicariant distributions (Fig. 1B): disjunct distributions which are known from Holocene subfossil material to have been continuous (Cameron *et al.*, 1996).

Geographical patterning is usually seen most clearly in Helicidae, which are in general far more abundant and diverse in open habitats than in the wetter and cooler laurel forests of Madeira. This is reflected in the greater number of helicid species (35) found alive on the overwhelmingly open and dry Porto Santo than on Madeira (30); the latter island is ca.18 times as large and has a far greater diversity of habitats.

EXTINCTIONS AND INTRODUCTIONS

We have two ways of estimating extinctions, the comparison of nineteenth and twentieth century records, and a comparison of subfossil and recent records.

On Porto Santo, only one species, the massive helicid *Pseudocampylaea lowei*, appears to have become extinct in this century. Even in the nineteenth century it was known alive from one locality only (it is abundant as a subfossil, but no dates are available).

Our own survey of Porto Santo conducted over a three-week period in 1993 revealed all but two of the remaining species reliably identified by Wollaston, and these two have also been found recently (M. Seddon, pers. comm.).

Where Wollaston (1878) gave sufficient detail, most species appear to have maintained their distribution and abundance, but a few which were found in lowland, sandy areas appear to have retreated. Conversely, the introduced *Theba pisana* has expanded its distribution, now being found in nearly all locations with any sand in the soil.

The situation on Madeira is more complicated. A number of species cataloged by Wollaston were known from only one or a very few sites, and these are not always relocatable with certainty. The island has not been resurveyed at the intensity involved on Porto Santo.

Of the 80 endemic species recorded alive at any time described for Madeira, our recent surveys of eastern and northern Madeira have revealed 56. Of the 24 not recorded by us, five were unknown in the nineteenth century, nine have certainly been found by others in recent years, but ten appear not to have been found during this century

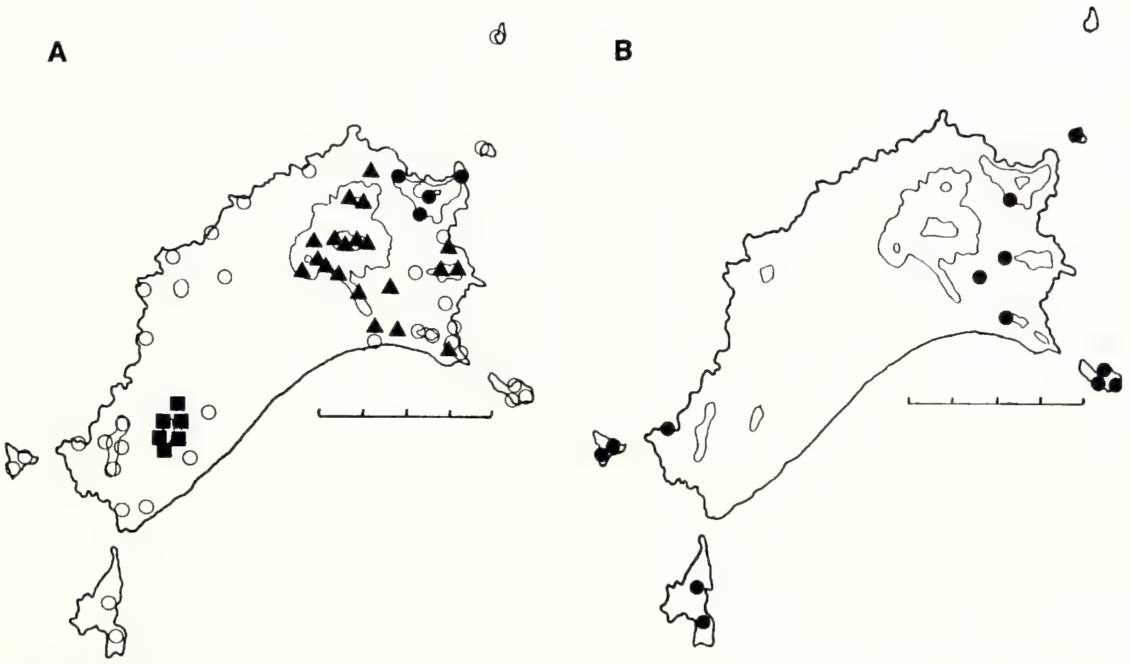

Fig. 1. Distributions of endemic species on Porto Santo. A. *Discula echinulata* (●), *D. bicarinata* (▲), and *D. leacockiana* (■). Other sites sampled shown as open circles. B. *Pseudocampylaea portosanctana.* Contours shown at 200 and 400 m.

(Groombridge, 1992). Many of these ten were very localized in the nineteenth century; five of them belong to the genus *Leiostyla* - small and often cryptic. It is unlikely that all of them are genuinely extinct; we suggest that, at a maximum, ca.10% of endemic species known to Wollaston have died out in the last 120 years, and that all of them were very rare before that.

At least two species, *Actinella arridens* and *Janulus stephanophora*, appear to have become very much rarer in the last 100 years; no explanation occurs to us. The southwest of Madeira, subject to heavy disturbance, has not been surveyed in detail; other declines could have occurred there.

Despite this low overall extinction rate, some areas have clearly become impoverished. Sandy areas on both islands have depauperate endemic faunas, and high densities of the introduced *Theba pisana*. On Madeira, planted conifer forests are virtually devoid of snails (Cook *et al.*, 1972) and some other areas dominated by introduced mimosas are depauperate (Cook *et al.*, 1990). Conversely, some open country endemics, for example *Discula polymorpha* and *Actinella nitidiuscula*, appear to have colonized cleared areas with some success.

The good subfossil record enables us to estimate extinctions in another way. Table 1 shows the numbers of species known alive or only subfossil on both islands. The Helicidae are separated from other families for reasons given below. Taking both islands together, they have a higher apparent extinction rate than the other families ($\chi^2 = 5.54$, $p < 0.05$).

Interpretation, however, must take into account the chances of preservation in the deposits. Table 2 shows the proportions of species known in the fossil record which are now extinct, and the proportion of the total fauna known in subfossil condition. Helicidae are better represented in the deposits than other families, and a much higher proportion of Porto-Santan than Madeiran species occur as subfossils. Fossiliferous deposits occur all over Porto Santo; they are confined to a small and atypical part of Madeira. The proportion of subfossil species now extinct is similar on both islands.

Table 1. The numbers of species recorded alive at any time or found only as subfossils (extinct) in the endemic faunas of Madeira (a) and Porto Santo (b).

(a) Madeira	Helicidae	Other Families	Total
Living	30	50	80
Extinct	9	5	14
Total	39	55	94
% Extinct	23.1	9.1	14.9
(b) Porto Santo	**Helicidae**	**Other Families**	**Total**
Living	37	17	54
Extinct	12	3	15
Total	49	20	69
% Extinct	24.5	15.0	21.7

Table 2. The numbers of endemic species known in subfossil condition, their proportions of total faunas, and the extinction rate for species known as subfossils for Madeira (a) and Porto Santo (b).

(a) Madeira	Helicidae	Other Families	Total
Known Subfossil	22	20	42
% of all fauna	56.4	36.4	44.7
Extinct	9	5	14
% of Subfossil Extinct	40.9	25.0	33.3
(b) Porto Santo	**Helicidae**	**Other Families**	**Total**
Known Subfossil	43	11	54
% of all fauna	87.7	55.0	78.3
Extinct	12	3	15
% of Subfossil Extinct	27.9	25.0	27.8

In the case of Madeira, of the 14 extinct species known from the sand deposits, at least nine appear to have died out after human colonization (Goodfriend *et al.*, 1994). We are not yet able to offer figures for Porto Santo.

The number and proportion of recent non-endemic species is much higher on Madeira than on Porto Santo (Table 3) and a lower proportion of non-endemics than endemics are Helicidae. Most non-endemics have probably been introduced (those which are anthropophilic almost certainly) but some, particularly very small species, could be native. Three non-endemics (*Punctum pygmaeum, Plagyrona placida,* and *Vitrea contracta*) are found as subfossils in pre-colonization deposits on Madeira, and *V. contracta* and *Balea perversa* have an exclusively montane distribution on Porto Santo. A small number of non-endemics also occur regularly in laurel forests. All slugs are non-endemic (Rähle, 1992).

DISCUSSION

THE EXTENT AND TIMING OF EXTINCTIONS

As estimated by changes over the last 100-150 yrs, the rate of apparent extinction reported here is very low for oceanic islands (Solem, 1990a; Groombridge, 1992) and especially for those subject to intense human intervention. The archipelago was discovered and colonized more than 400 yrs before detailed descriptions of the fauna were made. Early accounts (explored in Machado, 1947; Prestage, 1966; Crosby, 1986; and Ferraz, 1986) indicated large scale destruction of natural habitats by fire, fellings,

Table 3. The numbers of endemic and non-endemic species reported alive on both islands. Of Madeiran non-endemics, 13 are restricted anthropophiles. Figures in parentheses show the effect of excluding them.

(a) Madeira

	Helicidae	Other Families	Total
Endemics	30	50	80
Non-Endemics	12 (6)	47 (40)	59 (46)
% Non-Endemics	28.6 (16.7)	48.5 (44.4)	42.4 (36.5)
Total	42 (36)	97 (90)	139 (126)

(b) Porto Santo

	Helicidae	Other Families	Total
Endemics	37	17	54
Non-Endemics	14	8	12
% Non-Endemics	9.8	32.0	18.2
Total	41	25	66

and introduced mammals, and substantial agricultural exports were being made before the end of the fifteenth century. Extinctions could therefore have occurred on a large scale prior to nineteenth century surveys.

The subfossil record, which spans this period, suggests a higher rate of extinction of ca. 30% on both islands. The evidence from dating in the case of Madeira (Goodfriend *et al.,* 1994) suggests that two-thirds of this occurred after human occupation.

The record on Porto Santo is remarkably complete. Most parts of the island are sandy or near sand. Living species not found in deposits are mostly small rock-dwellers. Pending detailed analysis and dating, we can estimate that 20-25% of the fauna was destroyed by human colonization, but that it has since stabilized.

The record on Madeira is less complete, and could be unrepresentative of the island as a whole. Living faunas in the area of the deposits are poorer in endemics than many elsewhere (Cook *et al.,* 1990). On the other hand, the deposits have yielded nearly half the endemic species known from the island. An overall extinction rate similar to that on Porto Santo must be our best estimate.

Even these levels of post-colonization extinction are considerably lower than those recorded for the much shorter period of European colonization on many Pacific islands. These islands undoubtedly suffered earlier extinctions also, as a consequence of disturbance caused by the first human settlers.

CAUSES OF EXTINCTION

There is no direct evidence as to the causes of extinction, of the kind available for *Partula,* achatinellids, and endodontids on Pacific islands (Hadfield, 1986; Solem, 1990b; Cowie, 1992a). Some introduced Zonitidae and Subulinidae are potential predators, and introduced rodents certainly eat both native and introduced species, but we cannot ascribe any extinctions to their presence.

There is likewise little evidence of competition between native and introduced species. Site diversities of endemic and non-endemic species are independent of one another (Cook, 1984) and non-endemics fill spaces in the shell size and shape distribution unoccupied by endemics (Cameron and Cook, 1989). In one instance, it is possible that competition has caused extinction: the extinct *Caseolus bowdichianus* was very abundant on sandy substrata on both islands before colonization. It has now been replaced by the introduced *Theba pisana* which is similarly abundant on sandy substrata (Goodfriend *et al.,* 1994).

Most of the indirect evidence we have points to habitat destruction as the primary cause of extinction. Human intervention caused instability in sand-based habitats, and these now have lower than average diversities on both islands (Cook *et al.,* 1990; Cameron *et al.,* 1996). On Madeira, regions dominated by planted conifers are nearly snail-free (Cook *et al.,* 1972), and areas dominated by other non-native vegetation are species-poor (Cook *et al.,* 1990).

DIVERSITY AND DURABILITY IN THE SURVIVING FAUNA

Both islands support numerous sites in which the fauna is dominated by endemic and native species, at diversity levels (5-20 species) which are not depauperate by world standards (Solem, 1984). On Porto Santo, site diversities in rocky habitats are almost identical to those reported for similar maquis and phrygana habitats in the Aegean region (Cameron *et al.*, unpub.).

Thus while there is evidence of damage, and indeed of extinctions, the present state of the archipelago's fauna appears to be much healthier than that in many other oceanic islands subject to extensive human intervention.

Comparison with accounts of extinctions in these other islands suggests that there could be significance in the fact that many Madeiran and Porto Santan species have evolved to live in rather dry, rocky, and open environments, rather than being arboreal or dependent on damp litter from native trees. Not only did this preadapt them to resist clearance, it is also these habitats which have been subject to the greatest fluctuations in size and continuity before human colonization, building up allopatric diversity, and tolerance of change.

It is also the case that substantial areas of native laurel forests survive on the northern side of Madeira. Where native forests survive on some Pacific islands, high levels of extinction have followed the introduction of molluscan, arthopod, and vertebrate predators (Solem, 1990b; Cowie, 1992b). Introduced predators have not had this effect here (see above), nor in the Gulf of Guinea (Gascoigne, 1994), where *Euglandina rosea* (Férussac, 1818), the introduced predatory snail which has devastated many Pacific island faunas, has not been introduced.

In general, the pattern seen on Madeira resembles that found in the Mediterranean region, where despite massive and prolonged human alterations of the environment, many natives (and some restricted endemics) have survived, with the addition of species spread by humans and associated with their activities (Mylonas, 1984). The Madeiran fauna is clearly of European rather than African origin (Waldén, 1983) and the same family, the Helicidae, dominates the faunas of both the archipelago and of southern Europe. Many species are adapted to rather dry, exposed environments.

This apparent durability does not justify complacency over conservation. Destruction of the habitats on one small hill on Porto Santo (an area of less than 0.5 km^2) would eliminate three endemic species, and the destruction of remaining laurel forest on Madeira would remove far more.

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LITERATURE CITED

Cameron, R. A. D. and L. M. Cook. 1989. Shell size and shape in Madeiran land snails: do niches remain unfilled? *Biological Journal of the Linnean Society* 36:79-96.

Cameron, R. A. D. and L. M. Cook. 1992. The development of diversity in the land snail fauna of the Madeiran archipelago. *Biological Journal of the Linnean Society* 46:105-114.

Cameron, R. A. D., L. M. Cook, and J. Hallows. 1996. Land snails on Porto Santo: adaptive and non-adaptive radiation. *Philosophical Transactions of the Royal Society of London, B* 351:309-327.

Clarke, B. and J. Murray. 1969. Ecological genetics and speciation in land snails of the genus *Partula. Biological Journal of the Linnean Society* 1:31-42.

Cook, L. M. 1984. The distribution of land molluscs in eastern Madeira and the Desertas. *In: World Wide Snails,* A. Solem and A. C. van Bruggen, eds. pp. 46-55. Brill, Leiden.

Cook, L. M. In press. Madeiran landsnails: habitat, isolation and evolution. *Biological Journal of the Linnean Society.*

Cook, L. M., R. A. D. Cameron, and L. A. Lace. 1990. Land snails of eastern Madeira: speciation persistence and colonization. *Proceedings of the Royal Society of London, B* 239:35-79.

Cook, L. M., G. A. Goodfriend, and R. A. D. Cameron. 1993. Changes in the land snail fauna of eastern Madeira during the Quaternary. *Philosophical Transactions of the Royal Society of London, B* 339:83-103.

Cook, L. M., T. Jack, and C. W. A. Pettitt. 1972. The distribution of land molluscs in the Madeiran archipelago. *Boletim do Museo municipal, Funchal* 26:5-30.

Cowie, R. H. 1992a. Evolution and extinction of Partulidae, endemic Pacific land snails. *Philosophical Transactions of the Royal Society of London, B* 335:167-191.

Cowie, R. H., ed. 1992b. The impact of alien species on island ecosystems: extended abstracts of a symposium, 30 May 1991, Honolulu, Hawaii, XVII Pacific Science Congress. *Pacific Science* 46:383-404.

Crosby, A.W. 1986. *Ecological Imperialism: The Biological Expansion of Europe, 900-1900.* Cambridge University Press, Cambridge. 380 pp.

Ferraz, M. de L. de Freitas. 1986. *A Ilha de Madeira sob o Dominio de Casa Senhorial do Infante D. Henrique e seus descendentes.* Região Autonomia da Madeira, Funchal. 79 pp.

Gascoigne, A. 1994. The biogeography of land snails in the islands of the Gulf of Guinea. *Biodiversity and Conservation* 3:784-807.

Gittenberger, E. 1991. What about non-adaptive radiation? *Biological Journal of the Linnean Society* 43:263-272.

Goodfriend, G. A. 1987. Evaluation of amino acid racemization/epimerization dating using radiocarbon-dated fossil land snail shells. *Geology* 15:698-700.

Goodfriend, G. A. 1991. Patterns of racemization and epimerization of amino acids in land snail shells over the course of the Holocene. *Geochimica et Cosmochimica Acta* 55:293-302.

Goodfriend, G. A., R. A. D. Cameron, and L. M. Cook. 1994. Fossil evidence of recent human impact on the land snail fauna of Madeira. *Journal of Biogeography* 21:309-320.

Goodfriend, G. A., R. A. D. Cameron, L. M. Cook, M. A. Courty, N. Federoff, A. Kaufman, E. Livett, and J. Tallis. 1996. The Quaternary eolian sequence of Madeira: stratigraphy, chronology and paleoenvironmental interpretation. *Palaeogeography, Palaeoclimatology and Palaeoecology* 120:195-234.

Groh, K. and J. Hemmen. 1984. Revision der Gattung *Boettgeria* O. Boettger 1863. *Archiv für Molluskenkunde* 116:183-217.

Groh, K. and J. Hemmen. 1986a. Zur Kenntnis der Vitriniden des Madeira-Archipels (Pulmonata, Vitrinidae). *Archiv für Molluskenkunde* 115:1-39.

Groh, K. and J. Hemmen. 1986b. *Geomitra (Serratorotula) gerberi* n. subgen. n. sp. aus dem Quatär von Porto Santo (Pulmonata: Helicidae). *Archiv für Molluskenkunde* 117:33-38.

Groombridge, B., ed. 1992. *Global Biodiversity: Status of the Earth's Living Resources.* Chapman and Hall, London. 585 pp.

Hadfield, M. G. 1986. Extinction in Hawaiian achatinelline snails. *Malacologia* 27:67-81.

Hemmen, J. and K. Groh. 1985. Eine neue Art der Gattung *Geomitra* Swainson auf Porto Santo (Pulmonata: Helicidae). *Archiv für Molluskenkunde* 116:73-80.

Holyoak, D. T. and M. B. Seddon. 1986. An undescribed *Leiostyla* (Gastropoda: Pupillidae) from Madeira. *Journal of Conchology* 31:191-193.

Machado, J. F., ed. 1947. *Descobrimento da Ilha da Madeira e discorso da Vida e Feitos dos Capitaes da dita Ilha (Jeronimo Dias Leite, 1579).* University of Coimbra, Coimbra, Portugal. 137 pp.

Mitchell-Thomé, R. C. 1985. Radiometric studies in macaronesia. *Boletim Museo municipal, Funchal* 37:52-85.

Mylonas, M. 1984. The influence of man: a special problem in the study of the zoogeography of terrestrial molluscs on the Aegean Islands, Greece. *In: World Wide Snails,* A. Solem and A. C. van Bruggen, eds. pp. 249-260. Brill, Leiden.

Prestage, E. 1966. *The Portuguese Pioneers.* Black, London. 352 pp.

Rähle, W. 1992. Nacktschnecken (Arionidae, Milacidae, Agriolimacidae und Limacidae) von Madeira und Porto Santo (Mittelatlantische Inseln). *Malacologische Abhandlungen Staatliches Museum für Tierkunde, Dresden* 16:13-24.

Seddon, M. B. 1990. Undescribed Quaternary land-snail species from S. W. Porto Santo (Madeiran Islands) (Mollusca: Gastropoda).

Journal of Conchology 33:299-304.

Solem, A., 1983. *Endodontoid Land Snails from Pacific Islands (Mollusca: Pulmonata: Sigmurethra). Part II. Families Punctidae and Charopidae, Zoogeography.* Field Museum of Natural History, Chicago. 336 pp.

Solem, A. 1984. A world model of land snail diversity and abundance. *In: World Wide Snails,* A. Solem and A. C. van Bruggen, eds. pp. 6-22. Brill, Leiden.

Solem, A. 1990a. Limitations of equilibrium theory in relation to land snails. *Atti dei Congresi Lincei* 85:97-116.

Solem, A. 1990b. How many Hawaiian land snails are left? and what we can do for them. *Bishop Museum Occasional Papers* 30:27-40.

Tomiyama, K. and T. Kurozumi. 1992. [Terrestrial mollusks and conservation of their environment in the Ogasawara Islands.] *Regional Views* 5:39-81. (In Japanese with English summary)

Waldén, H. W. 1983. Systematic and biogeographical studies of the terrestrial gastropods of Madeira, with an annotated check-list. *Annales Zoologici Fennici* 20:265-275.

Wollaston, T. V. 1878. *Testacea Atlantica.* Reeve, London. 588 pp.

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APPENDIX

Three lists are given here: (a) species known only in subfossil condition, (b) endemic species recorded alive at some time, and (c) non-endemic species recorded alive. There are, inevitably, cases of taxonomic uncertainty and of difficulties in interpreting the status of records. Minor disagreements in categorization do not affect our general conclusions, and so we have not annotated the lists, which should not be regarded as definitive accounts of the fauna.

(a) Species known only as subfossils, presumed extinct (all endemic).

Madeira

Helicidae:
Geomitra delphinula (Lowe, 1831)
G. watsoni (Johnson, 1897)
Caseolus bowdichianus (Férussac, 1832)
C. sphaerulus (Lowe, 1854)
C. abjectus (Lowe, 1831)
C. subcalliferus (Lowe in Pfeiffer, 1859)
Actinella arcinella (Lowe, 1854)
A. promontoriensis Walden, 1983

Others:
Leiostyla wollastoni (Paiva, 1866)
Amphorella grabhami (Pilsbry, 1908)
Cylichnidia cylichna (Lowe, 1852)
Boettgeria lorenziana Groh and Hemmen, 1984
Phenacolimax crassus Groh and Hemmen, 1986

Porto Santo

Helicidae:
Geomitra gerberi Groh and Hemmen, 1986
G. acarinata Hemmen and Groh, 1985
Caseolus bowdichianus (Férussac, 1832)
C. baixoensis Walden, 1983
Actinella morenensis Seddon, 1990
A. crassiuscula (Cockerell, 1922)
A. arcinella (Lowe, 1854)
Spirorbula latinea (Paiva, 1866)
Discula echinoderma (Wollaston, 1878)
D. cockerelli (Noronha, 1923)
Leptaxis chrysomela (Pfeiffer, 1846)
L. psammophora (Lowe, 1852)

Others:
Craspedopoma mucronatum (Lowe, 1830)
Leiostyla espigaoensis Seddon, 1990
L. subcorneocostata Seddon, 1990

(continued)

Appendix (continued)

(b) Endemic species found alive. (*, also found as subfossils; +, not found alive in twentieth century).

Madeira

Helicidae:
*_Heterostoma paupercula_ (Lowe, 1831)
*_Geomitra tiarella_ (Webb and Berthelot, 1833)
G. moniziana (Paiva, 1867)
+_G. delphinuloides_ (Lowe, 1860)
Spirorbula latens (Lowe, 1852)
*_S. squalida_ (Lowe, 1852)
*_Caseolus compactus_ (Lowe, 1831)
C. leptostictus (Lowe, 1831)
Disculella compar Lowe, 1831
D. spirulina Cockerell, 1921
Actinella lentiginosa (Lowe, 1831)
*_A. actinophora_ (Lowe, 1831)
A. arcta (Lowe, 1831)
A. fausta (Lowe, 1831)
A. carinofausta Walden, 19983
A. robusta (Wollaston in Pilsbry, 1894)
A. arridens (Lowe, 1831)
*_A. obserata_ (Lowe, 1854)
A. armitageana Lowe, 1854
*_A. nitiduscula_ (Sowerby, 1824)
A. giramica (Lowe, 1852)
A. anaglyptica (Reeve, 1852)
*_Leminiscia calva_ (Lowe, 1831)
+_L. galeata_ (Lowe, 1860)
*_Discula polymorpha_ (Lowe, 1831)
*_Leptaxis erubescens_ (Lowe, 1831)
*_L. furva_ (Lowe, 1831)
*_L. membranacea_ (Lowe, 1852)
*_L. undata_ (Lowe, 1831)

Others:
Craspedopoma neritoides (Lowe, 1860)
*_C. mucronatum_ (Lowe, 1830)
C. lyonnetanum (Lowe, 1852)
C. monizianum (Lowe, 1860)
*_C. trochoideum_ (Lowe, 1860)
Columella microspora (Lowe, 1852)
*_Truncatellina linearis_ (Lowe, 1852)
*_Staurodon saxicola_ (Lowe, 1852)
Leiostyla cheilogona (Lowe, 1831)
L. vincta (Lowe, 1852)
L. irrigua (Lowe, 1852)
L. loweana (Wollaston, 1878)
+_L. cassidula_ (Lowe, 1852)
L. concinna (Lowe, 1852)
*_L. laurinea_ (Lowe, 1852)
*_L. sphinctostoma_ (Lowe, 1831)
L. arborea (Lowe, 1854)
L. simulator (Pilsbry, 1923)
L. fusca (Lowe, 1852)
+_L. laevigata_ (Lowe, 1852)
L. recta (Wollaston, 1878)
*_L. millegrana_ (Lowe, 1852)
+*_L. abbreviata_ (Lowe, 1852)
*_L. cassida_ (Lowe, 1831)
+*_L. lamellosa_ (Lowe, 1852)
+*_L. gibba_ (Lowe, 1852)

Porto Santo

Helicidae:
*_Heterostoma paupercula_ (Lowe, 1831)
*_Geomitra coronata_ (Deshayes in Férussac, 1819)
*_Spirorbula obtecta_ (Lowe, 1831)
*_S. depauperata_ (Lowe, 1831)
*_Caseolus compactus_ (Lowe, 1831)
*_C. consors_ (Lowe, 1831)
*_C. commixtus_ (Lowe, 1854)
*_C. sphaerulus_ (Lowe, 1854)
*_C. abjectus_ (Lowe, 1831)
*_C. subcalliferus_ (Lowe in Pfeiffer, 1859)
*_C. calculus_ (Lowe, 1854)
*_C. hartungi_ (Albers, 1852)
*_C. punctulatus_ (Sowerby, 1824)
*_C. solidus_ (Lowe, 1831)
*_Actinella effugiens_ Walden, 1983
Lemniscia michaudi (Deshayes, 1831)
*_Discula bicarinata_ (Sowerby, 1824)
D. echinulata (Lowe, 1831)
*_D. leacockiana_ (Wollaston, 1878)
*_D. oxytropis_ (Lowe, 1831)
D. turricula (Lowe, 1831)
*_D. cheiranticola_ (Lowe, 1831)
*_D. calcigena_ (Lowe, 1831)
*_D. tectiformis_ (Wood, 1828)
D. albersi Lowe, 1852
*_D. bulverii_ (Wood, 1828)
*_D. rotula_ (Lowe, 1831)
*_D. pulvinata_ (Lowe, 1831)
*_D. attrita_ (Lowe, 1852)
*_D. testudinalis_ (Lowe, 1852)
Leptaxis erubescens (Lowe, 1831)
*_L. wollastoni_ (Lowe, 1852)
*_L. nivosa_ (Sowerby, 1824)
*_Pseudocampylea portosanctana_ (Sowerby, 1824)
*+_P. lowei_ (Férussac, 1835)
*_Lampadia webbiana_ (Lowe, 1831)
*_Helix subplicata_ Sowerby, 1825

Others:
*_Leiostyla corneocostata_ (Wollaston, 1878)
L. relevata (Wollaston, 1878)
L. ferraria (Lowe, 1852)
L. degenerata (Wollaston, 1878)
L. monticola (Lowe, 1831)
*_L. calathiscus_ (Lowe, 1831)
Eucobresia media (Lowe, 1854)
*_Cecilioides eulima_ (Lowe, 1854)
*_Amphorella melampoides_ (Lowe, 1831)
*_A. triticea_ (Lowe, 1854)
*_A. oryzae_ (Lowe, 1852)
A. tuberculata (Lowe, 1852)
A. cimensis Walden, 1983
A. gracilis (Lowe, 1831)
A. terebella (Lowe, 1852)
*_Cylichnidia ovuliformis_ (Lowe, 1831)
Boettgeria lowei (Albers, 1852)

(continued)

Appendix (continued)

L. filicum Holyoak and Seddon, 1986
Lauria fanalensis (Lowe, 1852)
Hemilauria limneana (Lowe, 1852)
+_Discus guerinianus_ (Lowe, 1852)
+_D. defloratus_ (Lowe, 1854)
Phenacolimax nitidus (Gould, 1848)
*_P. marcidus_ (Gould, 1848)
P. riuvensis (Lowe, 1831)
P. behnii (Lowe, 1851)
P. albopalliatus Groh and Hemmen, 1986
*_Janulus bifrons_ (Lowe, 1831)
*_J. stephanophora_ (Deshayes, 1835)
+_Cecilioides eulima_ (Lowe, 1854)
Amphorella tornatellina (Lowe, 1831)
*_A._ cf. _minor_ (Wollaston, 1878)
A. mitriformis (Lowe, 1852)
A. producta (Lowe, 1852)
A. iridescens (Wollaston, 1878)
Pyrgella leacockiana (Lowe, 1852)
Boettgeria deltostoma (Lowe, 1831)
B. depauperata (Lowe, 1854)
B. obesiuscula (Lowe, 1863)
B. exigua (Lowe, 1831)
B. crispa (Lowe, 1831)

(c) Non-endemic species (all recorded alive). (* = very restricted or strictly anthropophile).

Madeira

Helicidae:
*_Candidula intersecta_ (Poiret, 1801)
*_Cernuella virgata_ (da Costa, 1778)
C. vestita (Rambur, 1868)
*_Helicella conspurcata_ (Draparnaud, 1801)
Cochlicella acuta (Müller, 1774)
C. barbara (Linné, 1758)
Caracollina lenticula (Michaud, 1831)
Theba pisana (Müller, 1774)
*_Otala lactea_ (Müller, 1774)
*_Cepaea nemoralis_ (Linné, 1758)
Helix aspersa Müller, 1774

Others:
Carychium tridentatum (Risso, 1826)
C. minimum Müller, 1774
Cochlicopa lubrica (Müller, 1774)
C. lubricella (Porro, 1838)
Columella aspera Walden, 1966
Vertigo pygmaea (Draparnaud, 1801)
Lauria cylindracea (da Costa, 1778)
Discocharopa aperta (Moellendorff, 1888)
Vallonia costata (Müller, 1774)
V. pulchella (Müller, 1774)
V. excentrica Sterki, 1893
Acanthinula aculeata (Müller, 1774)
Plagyrona placida (Shuttleworth, 1852)
Punctum pygmaeum (Draparnaud, 1801)
Toltecia pusilla (Lowe, 1831)
Helicodiscus singleyanus (Pilsbry, 1890)
*_Discus rotundatus_ (Müller, 1776)
*_Hawaiia minuscula_ (Binney, 1840)

Porto Santo

Helicidae:
Cochlicella acuta (Müller, 1774)
C. barbara (Linné, 1758)
Caracollina lenticula (Michaud, 1831)
Theba pisana (Müller, 1774)

Others:
Vitrea contracta (Westerlund, 1871)
Cecilioides acicula (Müller, 1774)
Rumina decollata (Linné, 1758)
Balea perversa (Linné, 1758)
Oxychilus alliarius (J. S. Miller, 1822)
Testacella maugei Férussac, 1819
Milax gagates (Draparnaud, 1801)
Lehmannia valentiana (Férussac, 1823)

(continued)

Appendix (continued)

Vitrea contracta (Westerlund, 1871)
Nesovitrea hammonis (Stroem, 1765)
Oxychilus draparnaudi (Beck, 1837)
O. alliarius (J. S. Miller, 1822)
**O. helveticus* (Blum, 1881)
Zonitoides arboreus (Say, 1816)
**Z. nitidus* (Müller, 1774)
Euconulus fulvus (Müller, 1774)
Cecilioides acicula (Müller, 1774)
C. nyctelia (Bourguignat, 1856)
Ferussacia folliculus (Gronovius, 1781)
Subulina striatella (Rang, 1831)
Rumina decollata (Linné, 1758)
Testacella maugei Férussac, 1819
**T. haliotidea* Draparnaud, 1801
Arion lusitanicus Mabille, 1868
A. hortensis Férussac, 1819
A. intermedius (Normand, 1852)
A. pascalinus Mabille, 1868
Milax gagates (Draparnaud, 1801)
**M. sowerbyi* (Férussac, 1823)
Deroceras laeve (Müller, 1774)
D. panormitanum (Lessona and Pollonera, 1882)
D. lombricoides (Simroth, 1891)
D. reticulatum (Müller, 1774)
Lehmannia valentiana (Férussac, 1823)
Limax flavus Linné, 1758
L. maximus (Linné, 1758)

Diversity and decline of land snails on Rota, Mariana Islands

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Abstract: This study reviews the land snail fauna of Rota to assess the diversity of the fauna (based on recent field collections and the literature) and the status of each species encountered (currently extant vs. apparently extinct but known from historically collected live specimens vs. known only from dead material). This information can be used to determine the conservation needs of land snails on Rota and to provide insights into the origin and evolutionary history of the fauna. Two known land snail predators have been introduced onto Rota since World War II: the predatory flatworm *Platydemus manokwari* De Beauchamp, 1962, and the predatory gastropod *Gonaxis kibweziensis* (Smith, 1894). Of the 43 species encountered on Rota, 9% are considered surviving well, 23% have questionable survival status, 56% are in decline, and 12% are potentially extirpated. Mariana Island land snails are more widespread among the islands of the archipelago than Hawaiian land snails. Single island endemics constitute 87% of the Oahu fauna but at most 31% of the Rota fauna. The more widespread distribution of Mariana Island land snails could be related to the frequent typhoons that track across the island group. It appears that speciation within the Mariana Islands has occurred at least in the families Assimineidae, Charopidae, and Partulidae.

Terrestrial gastropods are possibly the most extinction prone organisms on oceanic islands (Hadfield *et al.*, 1993; Paulay, 1994). Many insular land snails have restricted ranges and small population sizes, making them especially sensitive to introduced competitors, predators, and habitat destruction (Hadfield *et al.*, 1993). Also, certain species have been over-collected by shell enthusiasts in the past (Hadfield, 1986; Solem, 1990). Because many species of land snails on oceanic islands evolved *in situ*, under limited predation pressures, they tend also to possess few adaptations protecting them from introduced predators (Cowie, 1992).

Two known land snail predators have been introduced on Rota since World War II: the predatory flatworm *Platydemus manokwari* De Beauchamp, 1962, and the predatory gastropod *Gonaxis kibweziensis* (see Eldredge and Smith, 1994). The predatory gastropod *Euglandina rosea* (Férussac, 1818) has been introduced onto Guam, however, *E. rosea* was not found on Rota during 1994, 1995, or 1996. Neither is there any indication in the literature of the occurrence of this species on the island. These three predators, especially *P. manokwari* and *E. rosea*, have been implicated in declines of land snails on several Mariana Islands, as well as on other islands around the Pacific (Hadfield and Mountain, 1980; Clarke *et al.*, 1984; Murray *et al.*, 1988; Hopper and Smith, 1992).

To fully understand the evolution and biogeography of insular faunas, it is important to examine the diversity and distribution of the organisms prior to the onset of anthropogenic activity (Balouet and Olson, 1989). The only way to do this is to sample past faunas through the fossil record. On islands the best preserved fossil faunas typically are of land snails and vertebrates. Erroneous biogeographical and evolutionary theories can be drawn from data collected only from recent faunal and floral surveys (Steadman, 1993).

In the Mariana Islands, the Partulidae have been extensively studied (Crampton, 1925; Kondo, 1970; Hopper and Smith, 1992; Smith, 1993) while other land snails have received little attention aside from taxonomic studies (Quadras and Moellendorf, 1894a, b; Baker, 1938; Abbott, 1949; Cooke and Kondo, 1961; Solem, 1983; Kurozumi, 1994). While Guam was extensively surveyed for land snails in the late nineteenth century by J. F. Quadras (Quadras and Moellendorff, 1894a, b), the other islands in the archipelago were little studied before the 1920s. Crampton and Kondo made notable land snail collections on Rota in 1925, 1949, and 1952. While numerous species have been described and recorded from Guam, few records have been published from other islands in the archipelago.

The objectives of this study were to review the land snail fauna of Rota, specifically to determine (1) the diversity of the fauna, based on recent field collections and the literature, and (2) the current status of each species (extant, apparently extinct but known from historically collected live specimens, or known only from dead material). On the basis of these data I will evaluate the conservation status of the fauna, as well as its origin and evolutionary history.

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MATERIALS AND METHODS

Rota is the second southernmost island in the Mariana Islands, just north of Guam (Fig. 1). During July 1994, March 1995, and April 1996, I searched for and collected subfossil and living land snails at 26 sites on Rota (Fig. 2). Land snails other than partulids were collected at 14 of the 26 sites surveyed. The July 1994 collections were from paleontological excavations at Payapai Cave and As Matmos Cliffside Cave (sites 20 and 12 respectively). These sites along with a preliminary list of vertebrate remains from them, were described by Steadman (1992 and unpubl.). Land snails at these sites were picked from sediments sieved through *ca.* 1.6-mm mesh screens. The March 1995 survey focused on living land snails and associated land snail death assemblages (*i.e.* subfossils). This was part of a joint University of Guam and U. S. Fish and Wildlife Service survey of partulid tree snails on Rota; I was invited to survey other terrestrial snails. During April 1996, two days were spent searching for snails at two sites already studied during March 1995. At each site I searched microhabitats for live snails and also collected dead shells accumulated on the forest floor. Searching less accessible microhabitats, such as under bark and deep in rock cracks, was limited at some sites because of time constraints.

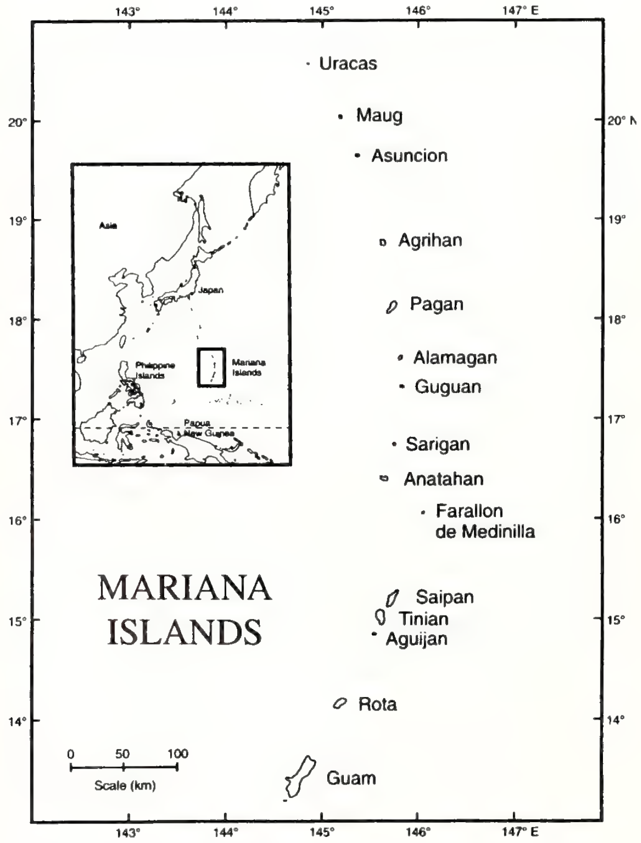


Fig. 1. Map of the Mariana Islands. Inset depicts the location of the Mariana Archipelago in the western Pacific region.

Voucher specimens of all species have been deposited in the University of Guam Invertebrate Collection, and where available in sufficient numbers, in the Bernice P. Bishop Museum (BPBM). Catalog numbers cited are from the Bishop Museum's Malacological Collection catalogs.

The status of each species (Table 1) was coded as follows. A species was considered "declined" if it was collected at < 5 sites as dead shells only or if collected at > 5 sites with ≤ 20% of the sites having live animals. A species was considered "possibly extirpated" on Rota if it was collected at ≥ 5 sites with no living animals found. A species was considered to be surviving well on Rota if it was collected at > 5 sites, with ≥ 50% of the sites having living populations. Species collected at < 5 sites with some sites supporting living populations and those collected at > 5 sites with 20-50% of the sites supporting living populations were considered as surviving with uncertain status.

I have used three designations to refer to species which are not definitely identified. Species names preceded by "cf." are represented by specimens that are close to the nominal form, but differ slightly from it. Species names preceded by "aff." are represented by specimens that differ sufficiently from the nominal taxon to be considered specifically distinct. Species names preceded by a "?" refer to specimens that were either too poorly preserved for definitive identification, or whose identity with the respective nominal taxon could not be fully ascertained.

RESULTS

Tables 1 and 2 summarize the status and collection information for each species on Rota. Additional details are treated in the systematic section below.

SYSTEMATIC REVIEW

Class GASTROPODA
Subclass PROSOBRANCHIA
Family HYDROCENIDAE

Solem (1988), Thompson and Dance (1983), and Thompson and Huck (1985) provided useful recent reviews of Pacific Hydrocenidae. Three species of hydrocenids have been described from the Mariana Islands; two of these are here recorded from Rota. These are the first published records of *Georissa* from Rota.

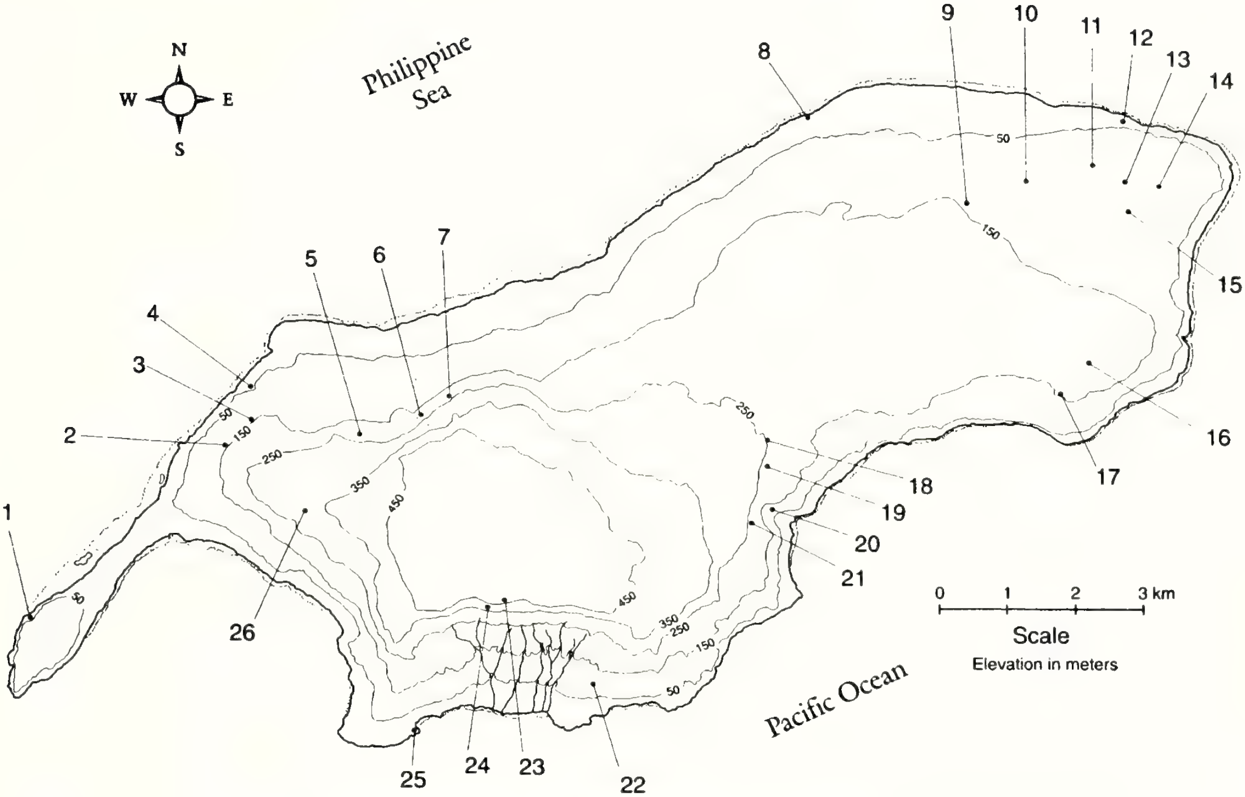


Fig. 2. Map of Rota indicating the locations of sites surveyed.

Genus *Georissa* Blanford, 1864
Georissa elegans Quadras and Moellendorff, 1894
Fig. 3

Shells of *Georissa elegans* have a sculpted and angled body whorl compared to the more rounded and weakly sculptured shell of *G. laevigata*. *G. elegans* was not found alive on Rota. *G. elegans* was described from Guam and figured by Zilch (1973a).

Georissa laevigata Quadras and Moellendorff, 1894
Fig. 4

Georissa laevigata was described from Guam and figured by Zilch (1973a). *G. laevigata* is widespread and abundant on Rota. It was found among limestone rubble and on the undersides of rotting leaves.

Family DIPLOMMATINIDAE
Genus *Palaina* Semper, 1865
Palaina taeniolata Quadras and Moellendorff, 1894
Fig. 5

This species was described from Guam; Zilch (1953) figured the lectotype. Live animals are abundant and widespread on Rota. Animals are common on the undersides of rotting leaves and on limestone rubble in forests.

Family TRUNCATELLIDAE
Fig. 6

Truncatellids tend to occur in marginal marine habitats. This habitat type was not thoroughly searched on Rota in 1995. One colony of an unidentified truncatellid was discovered on Rota in 1988 at Poña Point (B. Smith, pers. comm.). Live snails were still present at this site in 1995. No other truncatellids were found alive although numerous fragments were discovered at three other sites. Poña Point is a limestone plateau raised *ca.* 7-10 m above sea level, and is covered with short grasses growing on limestone, which has very little soil or sand and is in the spray zone of large waves. The assimineids *Omphalotropis suturalis*, and *Omphalotropis* sp. 7 are microsympatric with this truncatellid species on Rota. The same truncatellid species and *O. suturalis* inhabit and co-occur in similar habitats on Guam.

Family ASSIMINEIDAE

The Assimineidae is the most diverse family of land snails in the Mariana Islands, with 25 species recorded from Guam (Smith, 1993). Lectotypes and holotypes of 22 species described by Quadras and Moellendorff (1894a, b) were illustrated by Zilch (1967). While only six species

Table 1. Range among the southern Mariana Islands and status of species found on Rota. Island occurrences (numbers refer to references) 1, Baker, 1938; 2, Cooke and Kondo, 1961; 3, Zilch, 1973a; 4, Zilch, 1953; 5, Zilch, 1973b; 6, Solem, 1983; 7, Kondo, 1970; 8, Abbott, 1949; 9, Smith, unpubl.; 10, Solem, 1988; 11, Tsuda, 1969; 12, Muniappan, 1983; 13, Lange, 1950; +, new record; cf., new record of cf. nominal species. Range: I, introduced; M, Mariana Islands; R, Rota endemic; W, wide ranging; +, new record; ?, range not known at this time. Status on Rota: D, declined; E, potentially extirpated; S, surviving; S?, surviving with questionable status.

	Island Occurences					
Species	Guam	Rota	Tinian	Saipan	Range	Status on Rota
Georissa elegans	3	+		13	M	D
G. laevigata	3	+			M	S
Palaina taeniolata	4	+		13	M	S?
Truncatellidae sp(p).	5	+		13	?	S?
?*Allepithema* sp. 1		+			R	S?
Omphalotropis cookei	8	+		8	M	D
O. elongatula	9	+			M	E
O. laevigata	9	+			M	D
O. octhogyra	9	+			M	D
O. semicostulata	9	+			M	D
O. submaritima	9	(cf.)		13	M	D
O. suturalis	9	+			M	S?
O. quadrasi	8	+			M	D
O. sp. 1		+			R	D
O. sp. 2		+			R	D
O. sp. 3		+			R	D
O. sp. 4		+			R	D
O. sp. 5		+			R	D
O. sp. 6	+	+			M	D
O. sp. 7		+			R	S?
Paludinella conica	8	+		8	M	S
Quadrasiella clathrata	9	+			M	D
?*Q.* sp. 1		+			R	D
Pythia scarabaeus	9	+		13	W	E
Pacificella ?variabilis	+	+			W, I?	S?
Lamellidea subcylindrica	2	2			M	D
L. microstoma	2	2	2	2	M	
Elasmias quadrasi	2	2	2	2	M	S
Gastrocopta sp(p).	10	+		13	I,?	E
Nesopupa sp(p).	9	+		13	I,?	D
Partula gibba	7	7	7	7	M	D
P. cf./aff. *gibba*		+			R	S?
Samoana fragilis	7	7			M	S?
Gonaxis kibweziensis		11			I	D
Subulina octona	9	+		13	I	S?
Succinea sp(p).	9	+		13	?	E
Achatina fulica	12	12	12	12	I	E
Himeroconcha sp. 1		+			R	D
H. sp. 2		+			R	D
Semperdon heptaptychius	6	+			M	D
S. rotanus	6	6			M	
S. sp. 1		+			R	D
Liardetia tenuisculpta		1		1	W	
L. doliolum	1	1	1		W	
L. sp(p).		+			?	S
Lamprocystis fastigata	1	1	1		M	
L. sp(p).		+		13	?	S?
?Genus ?species		+			I,?	D

were collected alive on Rota, an additional 13 were encountered as subfossils.

Genus *Allepithema* Tomlin, 1931

?Allepithema sp. 1

Fig. 7

This species is tentatively placed in *Allepithema*. It is distinct from the six species of *Allepithema* described from Guam by Quadras and Moellendorff (1894b), and is only known from Rota. A single living animal was found at each of two sites.

Genus *Omphalotropis* Pfeiffer, 1851

This is the most diverse genus of land snails on Rota and Guam. Smith (1993) listed 16 species from Guam; 15 species are here recorded from Rota.

Omphalotropis cookei Abbott, 1949

Fig. 8

Omphalotropis cookei is distinguishable from other Rota *Omphalotropis* by the presence of "pronounced spiral threads" (Abbott, 1949). Abbott (1949: 265) stated, "*O. cookei* is closest in morphological characters to *Omphalotropis erosa* (Quoy and Gaimard, 1832) from Guam," but that species was not found on Rota. *O. cookei* is closest morphologically to *O. elongatula*, on Rota.

Omphalotropis cookei was previously known from Guam and Saipan (Abbott, 1949). Although this species was found dead at seven sites, only a single living population was encountered on Rota. Live animals were collected from the undersides of decaying leaves. *O. cookei* appears to have declined on Rota.

Omphalotropis elongatula Quadras and Moellendorff, 1894

Fig. 9

Previously known only from Guam, *Omphalotropis elongatula* is closest to *O. cookei* on Rota (see above). No live animals of *O. elongatula* were found on Rota during 1994 or 1995.

Omphalotropis laevigata Quadras and Moellendorff, 1894

Fig. 10

Omphalotropis laevigata was described from Guam. Shells which nearly match figured specimens from Guam (Zilch, 1967) were found at As Matmos Cliffside Cave on Rota.

Omphalotropis octhogyra Quadras and Moellendorff, 1894

Fig. 11

Previously known only from Guam, *Omphalotropis octhogyra* was abundant as subfossils at one site on Rota. Many *O. octhogyra* shells collected at this site appeared to be fresh (still covered with periostracum). At this site only one land snail species was found alive *(Georissa elegans)*, although 14 other land snail species were found as subfossils in leaf litter and soil samples. Traces of the predatory flatworm, *Platydemus manokwari*, were noted at this site.

Omphalotropis semicostulata Quadras and Moellendorff, 1894

Fig. 12

This species was previously known only from Guam. No living animals were found.

Omphalotropis cf. *submaritima* Quadras and Moellendorff, 1894

Fig. 13

Omphalotropis cf. *submaritima* can be distinguished from the similar *O. suturalis* by its larger size and more rounded sutural ramps. Dead shells of *O.* cf. *submaritima* were found at one site on Rota. Previously this species was only known from Guam and Saipan.

Omphalotropis suturalis Quadras and Moellendorff, 1894

Fig. 14

Omphalotropis suturalis was described from Guam and is only known from two populations on Rota. This species appears to be restricted to coastal margins of forests; both on Guam (pers. obs.) and Rota.

Omphalotropis quadrasi Moellendorff, 1894

Fig. 15

Abbott (1949) redescribed and figured *Omphalotropis quadrasi*, then considered a Guam endemic. One fresh *O. quadrasi* shell was found at one site on Rota.

Omphalotropis "carinate" species complex (species 1-5)

Seventeen specimens collected as subfossils at two locations on Rota appear to represent a previously unrecognized species complex characterized by turriculate shells ornamented by a well-developed carina. All of the species appear to be undescribed and no species like them have been collected on Guam to date. They could represent an endemic radiation of assimineids on Rota. They are tentatively included in *Omphalotropis* on the basis of Thiele's (1929: 172) definition of the genus: "Shell oval to turriculate, with perforated umbilicus, surrounded by a more or less distinct ring; aperture oval, apertural margin in most cases interrupted, occasionally somewhat broadened." At present five species are recognized, but as material to evaluate variation is limited, this number might have to be revised in the future. All five putative species possess the generic characters mentioned by Thiele except apertural characters cannot be resolved in all specimens due to their poor preservation.

Table 2. Distribution and collection status of each species found on Rota. F, subfossil collection; FL, collected both subfossil and live; L, collected alive.

	SITE NUMBER													
SPECIES	1	2	4	6	7	8	10	11	12	17	20	21	24	25
Georissa elegans	F					F	F				F			
G. laevigata	F	FL	L	L	L	FL	F	F		FL	FL		L	
Palaina taeniolata	F	F	L	L		F	F	F	F	F	FL		L	
Truncatellidae						F			F		F			L
?Allepithema sp. 1				L							F		L	
Omphalotropis cookei	F	F	L			F		F		F	F			
O. elongatula	F	F				F	F			F	F			
O. laevigata									F					
O. octhogyra		F												
O. semicostulata						F					F			
O. cf. *submaritima*		F												
O. suturalis						FL			F		F			L
O. quadrasi												F		
O. sp. 1									F		F			
O. sp. 2									F		F			
O. sp. 3											F			
O. sp. 4											F			
O. sp. 5											F			
O. sp. 6	F	F		L			F			F	F			
O. sp. 7														L
Paludinella conica	FL	F	L	L	L	F	F		F	F	F	F	L	
Quadrasiella clathrata											F			
?Q. sp. 1											F			
Pythia scarabaeus	F	F				F			F	F	F	F	F	
Pacificella ?variabilis	FL								F		F			
Lamellidea subcylindrica	F	F				F			F	F	F		L	
Elasmias quadrasi	FL	F					F	FL	F	F	FL		L	
Gastrocopta sp(p).	F					F	F		F	F	F	F		
Nesupupa sp(p).	F										F			
Partula gibba	F	F	L	L	F	FL	F	F	F	F	FL		FL	
P. cf./aff. *gibba*									F		F			
Samoana fragilis													L	
Goniaxis kibweziensis			F											
Sublina octona	Encountered at many sites around Rota.													
Succinea sp(p).	F	F						F	F		F	F	F	
Achatina fulica	Encountered at many sites around Rota.													
Himeroconcha sp. 1								F		F				
H. sp. 2										F				
Semperdon heptaptychius						F				F				
S. sp. 1										F				
Charopid sp(p.) indet.		F		F				F						
Liardetia sp(p.)	FL		L			FL	F	FL		F	FL	FL	L	
Lamprocystis sp(p.)	FL	F		L		F	F		F	F	F		L	
?Genus ?species									F					

Omphalotropis sp. 1

Fig. 16

Shell small, globose, and with carina not reaching the apex. Known from 11 specimens from excavation at sites 12 and 20.

Omphalotropis sp. 2

Fig. 17

Shell similar to *Omphalotropis* sp. 1 but slightly larger with a higher, thinner spire. Known from one specimen each from excavations at sites 12 and 20.

Omphalotropis sp. 3

Fig. 18

Shell high spired, cyrtoconoid (with convex sides); suture channeled with a somewhat prominent carina. Site 20 yielded two specimens from excavations.

Omphalotropis sp. 4

Fig. 19

Shell slightly smaller and similar to *Omphalotropis* sp. 3, high spired, cyrtoconoid, with carina not as prominent. One specimen was collected from excavation at site 20.

Omphalotropis sp. 5

Fig. 20

Shell close to *Omphalotropis* sp. 4 but the carina is near the middle of a whorl. Known from one specimen from excavation at site 20.

Omphalotropis sp. 6

Fig. 21

Omphalotropis sp. 6 is conchologically close to *O. elongatula* and *O. cookei* on Rota and to *O. erosa* (Quoy and Gaimard, 1832) from Guam. *O.* sp. 6 was found living under limestone rubble in the forest, but not on the undersides of decaying leaves. They were moderately abundant.

Omphalotropis sp. 7

Fig. 22

This species was only found at Poña Point living sympatrically with *Omphalotropis suturalis* and a truncatellid. It is similar in shell shape to *O. suturalis* but differs by being finely ribbed. Of the three species found at Poña Point, *O.* sp. 7 is the rarest in my collections.

Genus *Paludinella* Pfeiffer, 1841

Paludinella conica (Quadras and Moellendorff, 1894)

Fig. 23

Paludinella can be separated from the similar genus *Assiminea* Fleming, 1828, by the absence of a fine spiral thread just below the suture (Abbott, 1949). *P. conica* is the most abundant and widespread assimineid on Rota. Living specimens occurred at five sites and were noted as common at many locations by U. S. Fish and Wildlife Officers on Rota. They were also found as subfossils at seven other sites.

Genus *Quadrasiella* Moellendorff, 1894

Quadrasiella was erected by Moellendorff in Quadras and Moellendorff (1894b) for two species of land snails on Guam; Moellendorff (1900) described a third species from Pohnpei (Caroline Islands). *Quadrasiella* is differentiated from other assimineids by its operculum, which has an "inner calcareous lamella which overlaps the peristome" (Moellendorff, 1900: 119).

Quadrasiella clathrata Moellendorff, 1894

Fig. 25

This species, previously known only from Guam, was figured by Zilch (1967). On Rota it was found in excavations at site 20.

?Quadrasiella sp. 1

Fig. 24

?Quadrasiella sp. 1 is conchologically similar to *Q. clathrata* from Guam. It differs from *Q. clathrata* in that the body whorl does not expand as fast, the spire is higher, and the sculpture is more prominent. No opercula were found in association with the new specimens, thus its generic status is uncertain. This species is known only from Rota.

Subclass PULMONATA

Order ARCHAEOPULMONATA

Family ELLOBIIDAE

Genus *Pythia* Röding, 1798

Pythia scarabaeus (Linné, 1758)

Pythia scarabaeus ranges widely on islands in the western Pacific. Shells of this species are a highly visible component of the ground shell paleofauna in many forested areas on Rota. No living animals were found. Bishop Museum records indicate that the species was common alive on Rota in 1925 and 1949 (BPBM 213232, 213261, and 82427). Subsequently *P. scarabaeus* has declined and could be extirpated on Rota.

Order STYLOMMATOPHORA

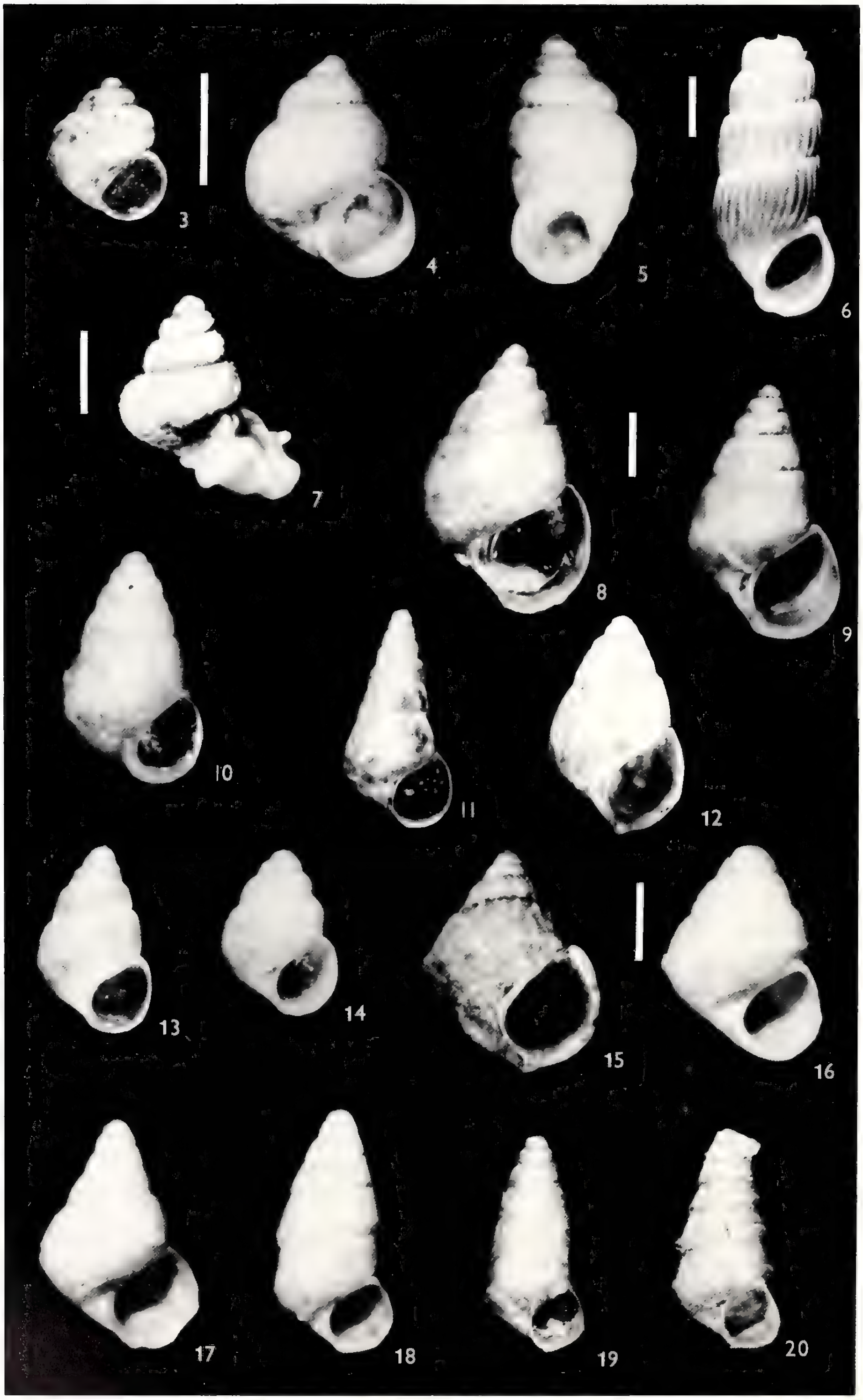
Family ACHATINELLIDAE

Genus *Pacificella* Odhner, 1922

Pacificella ?variabilis Odhner, 1922

Fig. 26

Species of *Pacificella* are widely distributed on Pacific islands and were recorded from the Mariana Islands without specific locality data (Cooke and Kondo, 1961; Preece, 1995). Its wide range indicates it could have been



Figs. 3-20. Land snails from Rota. Site numbers for each species are from Fig. 2 and are listed after the species name. **3.** *Georissa elegans*, 20. **4.** *G. laevigata*, 20. **5.** *Palaina taeniolata*, 12. **6.** Truncatellidae sp., 3. **7.** ?*Allepithema* sp. 1, 6. **8.** *Omphalotropis cookei*, 8. **9.** *O. elongatula*, 1. **10.** *O. laevigata*, 12. **11.** *O. octhogyra*, 26. **12.** *O. semicostulata*, 20. **13.** *O.* cf. *submaritima*, 12. **14.** *O. sutralis*, 25. **15.** *O. quadrasi*, 21. **16.** *O.* 'carina' sp. 1, 20. **17.** *O.* 'carina' sp. 2, 20. **18.** *O.* 'carina' sp. 3, 20. **19.** *O.* 'carina' sp. 4, 20. **20.** *O.* 'carina' sp. 5, 20. Scale bars = 1 mm except Fig. 4 = 2 mm. Fig. 1 scale bar also refers to Figs. 4, 5, and 10. Fig. 6 scale bar also refers to Figs. 12-14 and 18-20. Fig. 8 scale bar also refers to Figs. 9, 11, and 15. Fig. 16 scale bar also refers to Fig. 17.

introduced aboriginally onto the Mariana Islands. Live animals were found at one site on Rota, on the undersides of tree leaves.

Genus *Lamellidea* Pilsbry, 1910

Lamellidea microstoma (Quadras and Moellendorff, 1894)

Cooke and Kondo (1961) reviewed this species. It was not encountered during the present surveys. It was figured by Zilch (1962).

Lamellidea subcylindrica (Quadras and Moellendorff, 1894)

Fig. 27

This species is known only from Guam and Rota (Cooke and Kondo, 1961). Cooke and Kondo (1961) noted this to be a less abundant species than *Lamellidea microstoma* on both Guam and Rota. The second species of *Lamellidea* known in the Marianas *(L. microstoma)* was not found on Rota in 1994 or 1995. Live animals of *L. subcylindrica* were collected from the undersides of tree leaves.

Genus *Elasmias* Pilsbry, 1910

Elasmias quadrasi (Moellendorff, 1894)

Fig. 28

This species is distinguished from other achatinellids on Rota by its small size (*ca.* 2.5 mm length) and distinct apertural barriers. *Elasmias quadrasi* is known from Guam, Rota, Tinian, and Saipan and extends onto the northern Mariana Islands (Cooke and Kondo, 1961). Live animals were found aestivating on the undersides of leaves and twigs in trees.

Family PUPILLIDAE

Genus *Gastrocopta* Wollaston, 1878

Gastrocopta sp(p).

Fig. 29

Gastrocopta is a wide-ranging genus that apparently was in part distributed by humans (Solem, 1959, 1988). No living specimens were found on Rota. Records at the Bishop Museum indicate a species of *Gastrocopta* was alive on Rota in 1949 (BPBM 213220, 213221).

Genus *Nesopupa* Pilsbry, 1900

Nesopupa sp(p).

Fig. 30

Australian *Nesopupa* were reviewed by Solem (1988). No live *Nesopupa* were found on Rota in 1995. *Nesopupa (Nesopupa) quadrasi quadrasi* (Moellendorff, 1894) was described from Guam. A few records of live *Nesopupa* on Rota were found at the Bishop Museum (*e.g.* BPBM 82202, 82429), indicating that live specimens were collected in 1925 by H. G. Hornbostel. The wide range of many *Nesopupa* species are indicative of human transport. Specific identification of this material was not made.

Family PARTULIDAE

Genus *Partula* Férussac, 1821

Partula gibba Férussac, 1821

Fig. 31

Partula gibba is endemic to the Mariana Islands, ranging from Guam through the northern Mariana Islands (Kondo, 1970). This was a widespread species on Rota at one time attested by the large number of subfossils found at almost all sites visited and the large number of live-collected specimens housed at the Bishop Museum. Only five of the sites surveyed now support living populations of *P. gibba,* indicating that this species has declined greatly in recent years. A similar decline for *P. gibba* was documented by Hopper and Smith (1992) on Guam.

Partula cf./aff. *gibba* Férussac, 1821

Fig. 32

Four shells of a distinctive *Partula* were collected from paleontologic test pits at Payapai and As Matmos caves. They are close in general shell shape to *P. gibba*, but differ in having an extremely thickened and heavy shell. Similar shells were not mentioned in Crampton's (1925) monograph on variation in Partulidae of the Mariana Islands. Limited material leaves the status of these shells uncertain; they could represent an extinct undescribed (sub)species or local race of *P. gibba.*

Genus *Samoana* Pilsbry, 1909

Samoana fragilis (Férussac, 1821)

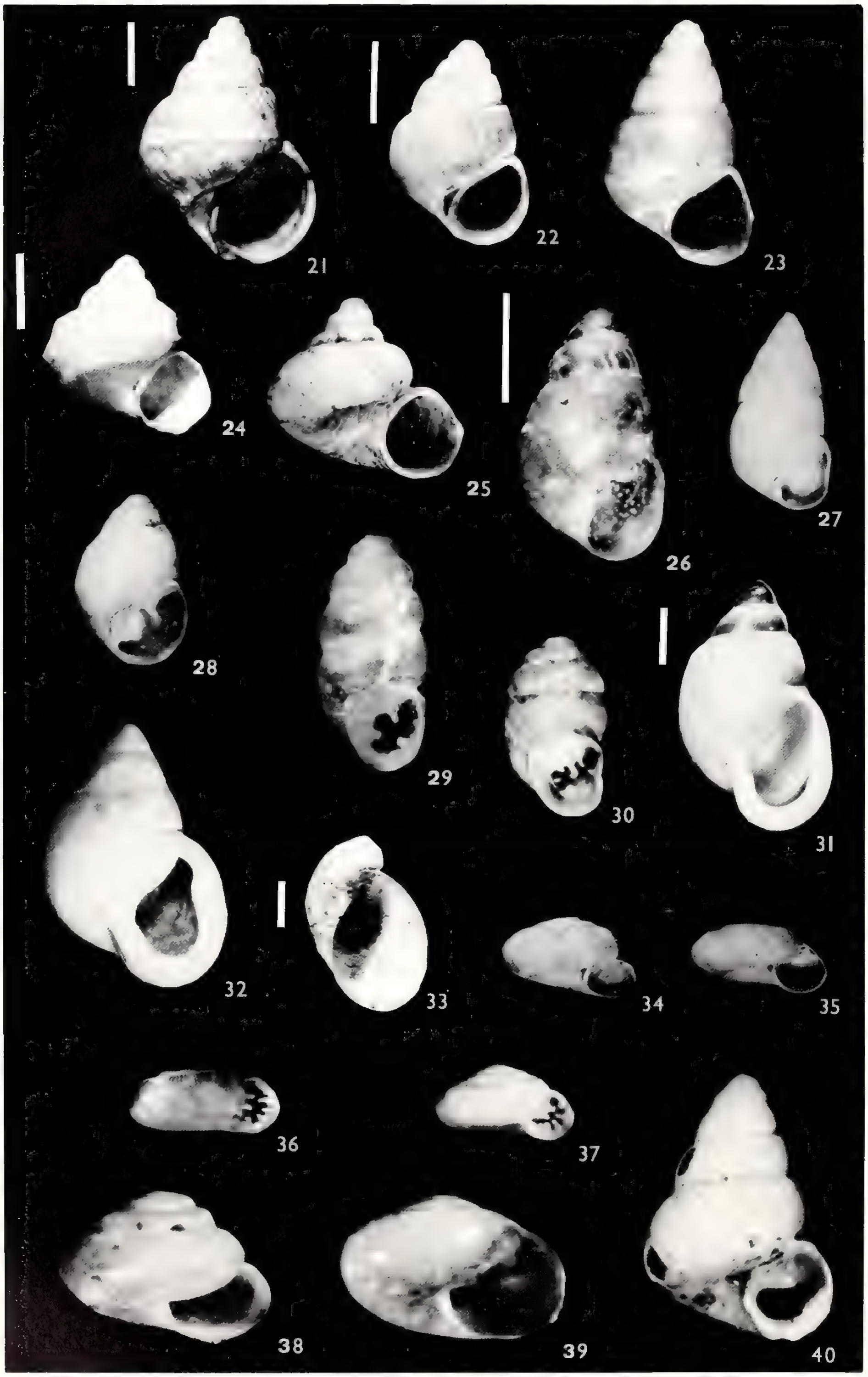
No shells of this species were found in 1995. Bishop Museum records indicate *Samoana fragilis* once occurred on Rota, on the Sabana in 1959 (BPBM 213164-213168). Today the Sabana area has been mostly converted into agricultural fields and no living partulids were found there in 1995. During a short field trip in 1996 to Rota, a colony of *S. fragilis* was found at site 24.

Family STREPTAXIDAE

Genus *Gonaxis* Taylor, 1877

Gonaxis kibweziensis (Smith, 1894)

This species was introduced to Rota probably from Aguijan where it was released as a possible control agent for *Achatina fulica*. Shells of this species were found at site 2. I know of no further information about this species on Rota other than it was alive in 1969 (Eldredge, 1969).



Figs. 21-40. Land snails from Rota. Site numbers for each species are from Fig. 2 and are listed after the species name. **21.** *Omphalotropis* sp. 6, 6. **22.** *O.* sp. 7, 25. **23.** *Paludinella conica*, 24. **24.** ?*Quadrasiella* sp. 1, 20. **25.** *Q. clathrata*, 20. **26.** *Pacificella ?variablis*, 20. **27.** *Lamellidea subcylindrica*, 20. **28.** *Elasmias quadrasi*, 20. **29.** *Gastrocopta* sp(p)., 12. **30.** *Nesopupa* sp(p)., 1. **31.** *Partula gibba*, 8. **32.** *P.* cf./aff. *gibba*, 20. **33.** *Succinea* sp(p)., 13. **34.** *Himeroconcha* sp. 1, 20. **35.** *H.* sp. 2, 20. **36.** *Semperdon heptaptychius*, 1. **37.** *S.* sp. 1, 20. **38.** *Liardetia* sp(p)., 1. **39.** *Lamprocystis* sp(p)., 1. **40.** ?Genus, 20. Scale bars = 1 mm except 31, 32 = 4 mm and 33 = 3 mm. Fig. 26 scale bar also refers to Figs. 29 and 30. Fig. 22 scale bar also refers to Figs. 23, 27, 34-37, and 39. Fig. 21 scale bar also refers to Figs. 25 and 28. Fig. 24 scale bar also refers to Figs. 38 and 40.

Family SUBULINIDAE
Genus *Subulina* Beck, 1837
Subulina octona (Bruguière, 1789)

Subulina octona, a human-transported species, was reviewed and figured by Solem (1988). On Rota it was noted alive at several sites, although I did not collect this species. It apparently is surviving well on the island. On many islands it is noted for inhabiting cultivated gardens.

Family SUCCINEIDAE
Genus *Succinea* Draparnaud, 1801
Succinea sp(p).
Fig. 33

The number of species of *Succinea* on Rota could not be determined because they cannot be separated easily by shell characters alone (Cowie *et al.*, 1995). Four species of *Succinea* were recorded from Guam (Smith, 1993), two of which were figured by Zilch (1978). Bishop Museum records indicate *Succinea* was widespread and living on the island in 1925 and 1949 (*e.g.* BPBM 213287-213289). *Succinea* might be extirpated from Rota.

Family ACHATINIDAE
Genus *Achatina* Lamarck, 1799
Achatina fulica Bowdich, 1822

Achatina fulica, an introduced agricultural pest, was once extremely common on Rota, demonstrated by the large numbers of shells seen at many sites (Muniappan, 1983). Several surveys were conducted during the late 1940s and early 1950s by Bishop Museum staff and associates to ascertain the status of *A. fulica* in the Mariana Islands (Lange, 1950; Mead and Kondo, 1950; Chamberlin, 1952; Kondo, 1952). The occurrence of this species was not tracked during 1994 and 1995 because its previous distribution on the island is known. No living animals or fresh shells were seen during surveys in 1994 or 1995. However, residual populations could still exist on Rota in agricultural areas, which were not surveyed during 1994 or 1995.

Family CHAROPIDAE

Solem's (1976, 1983) monographs provide the systematic framework for the Mariana Island charopids. No living specimens of any charopid were found during 1995 or 1996 surveys, although Bishop Museum records and records in Solem (1983) indicate they were once common. All Rota charopids could be extirpated. Many of the specimens collected were either fragmentary or too obscured by soil to make species identification possible (indicated on Table 2 as "charopid sp(p). indet.").

Genus *Himeroconcha* Solem, 1983
Himeroconcha sp. 1
Fig. 34

This apparently undescribed species appears to fit within *Himeroconcha* generic limits set by Solem (1983). It differs from *H. rotula* (Quadras and Moellendorff, 1894) by its rapidly descending body whorl. A single shell of this species was collected at each of two sites.

Himeroconcha sp. 2
Fig. 35

Eight subfossil specimens of another apparently undescribed *Himeroconcha* were found at site 12. Three complete specimens show this species to fall within the generic limits set for *Himeroconcha* by Solem (1983). The apertural periphery of *Himeroconcha* sp. 2 is expanded compared to that of *H. rotula*. The body whorl of *H.* sp. 2 does not descend as rapidly as in *H.* sp. 1.

Genus *Semperdon* Solem, 1983
Semperdon heptaptychius
(Quadras and Moellendorff, 1894)
Fig. 36

Shells of this species were found at two sites on Rota. Previously the species was known only from Guam (Solem, 1983).

Semperdon rotanus Solem, 1983

Semperdon rotanus was described and thoroughly reviewed by Solem (1983). It was not encountered during the present survey.

Semperdon sp. 1
Fig. 37

Semperdon sp. 1 is easily distinguished from other Rota *Semperdon* species by its extremely high spire. It was found at one site as rare subfossils.

Family HELICARIONIDAE
Genus *Liardetia* Gude, 1913
Liardetia sp(p).
Fig. 38

Two species of *Liardetia* were recorded from Rota by Baker (1938): *L. doliolum* (Pfeiffer, 1846) and *L. tenuisculpta* (Moellendorff, 1893). The identification of new material from my collections is uncertain at this stage. *Liardetia* specimens were collected from screw pine leaves and other vegetation by using a beating sheet. The genus seems to survive well on Rota and appears not in decline.

Genus *Lamprocystis* Pfeffer, 1883
Lamprocystis sp(p).
Fig. 39

One species of *Lamprocystis* was recorded from Rota by Baker (1938): *L. fastigata* (Gude, 1917). The identification of new material in my collections is uncertain at this stage. Living specimens were found on the underside of decaying leaves on the forest floor. At site 1, *Platydemus manokwari* was noted on the underside of a rotting leaf alongside four adults of *L.* sp(p). Very few living specimens of *L.* sp(p). were found on Rota, although they were fairly abundant at site 24.

Family ?
Genus ?
Fig. 40

The taxonomic affinity of this species is uncertain at present. It resembles the many Achatinellidae in that it has an unsculptured shell, with a strong and large parietal barrier present. It was found only at the paleontological excavation at Payapai Cave.

DISCUSSION

This is the first review of the land snail fauna of Rota. I have recorded a total of at least 43 species during the 1994, 1995, and 1996 surveys. Several taxa, however, were identified only to genus and could contain multiple species. Thus among helicarionids, I identified specimens only to the genera *Liardetia* and *Lamprocystis*; Baker (1938) recorded three helicarionid species from Rota (*Liardetia tenuisculpta, L. doliolum,* and *Lamprocystis fastigata*). The pupillids (Gastrocopta sp[p]. and *Nesopupa* sp[p].) and the truncatellid taxa were not identified to the species level in my collections. Two additional land snail species have been recorded in the literature from Rota which were not encountered in the present survey: *Lamellidea microstoma* (Achatinellidae) and *Semperdon rotanus* (Charopidae) (Cooke and Kondo, 1961; Solem, 1983).

This brings the total native and introduced fauna of Rota to at least 46 taxa, noticeably fewer than the *ca.* 74 species known from Guam. This difference possibly reflects in part the much lower intensity of sampling that Rota has received compared with Guam, but could also be due to the island's smaller size and greater habitat homogeneity.

Three species (*Subulina octona, Achatina fulica,* and *Gonaxis kibweziensis*) are certainly introductions while three others (*Nesopupa* sp[p]., *Gastrocopta* sp[p]., and *Pacificella ?variabilis*) are potential introductions (Cook and Kondo, 1961; Solem, 1988). One additional species remains unidentified even to family and is not considered further below. This leaves at least 39 taxa as indigenous to Rota.

Eighty-five percent of the indigenous species appear to be endemic to the Mariana Islands. Of the three indigenous species that are known to be more widespread, *Liardetia doliolum* and *L. tenuisculpta* are recorded from the Philippines and the Caroline Islands while *Pythia scarabaeus* is widespread throughout western Pacific islands (Baker, 1938).

Thirty-six percent of the Mariana endemic taxa are known from Rota only. These are possible Rota endemics. Guam is the closest island to Rota and also has the best known modern land snail fauna of any Mariana Island. However, Guam's fossil fauna is still poorly known, and some of the species recorded as Rota endemics might have existed on Guam in the past.

Restriction of species ranges to single islands is characteristic of land snails and many insect groups (*e. g.* weevils) on central Pacific islands (Crampton, 1925; Baker, 1938; Cooke and Kondo, 1961; Paulay, 1994). As noted above, most of the Rota land snails occur on neighboring islands also (*i.e.* Guam and anecdotally from Tinian and Saipan). Within the Mariana Islands, wide multi-island species ranges have been found to be the rule among the systematically revised families Achatinellidae, Partulidae, and Helicarionidae (Baker, 1940; Cooke and Kondo, 1961; Kondo, 1970). The other taxonomically well-known family, the Charopidae, has not been extensively collected on the Mariana Islands outside of Guam. For other historically recorded non-introduced families (Hydrocenidae, Diplommatinidae, Truncatellidae, Assimineidae, Ellobiidae, and Succineidae) little is known about the ranges of component species among the southern Mariana Islands. Observing that many land snail species are widely distributed among the Mariana Islands implies the existence of an effective dispersal mechanism.

To evaluate whether the proportion of single island endemics is indeed relatively low in the Mariana Islands compared with other Pacific islands, I compared the proportion of single island endemics on Rota and on Oahu (Hawaiian Islands). The fauna of the Hawaiian Islands was chosen for comparison, because it is taxonomically well known at the entire faunal level (Cowie *et al.*, 1995; Cowie, 1996). Oahu was chosen as a representative island from that archipelago.

A much larger proportion of Oahu's (87%, $N = 283$) than of Rota's (31%, $N = 39$) indigenous land snail fauna is constituted by single island endemics ($G = 54.5$; $p << 0.001$). The greater proportion of widespread species in the Marianas than in the Hawaiian Islands could be the result of several factors. A larger proportion of Rota's fauna is comprised of prosobranchs, which might be better dispersers by virtue of the protection provided by their operculum. Thus while 59% of the indigenous land snails of Rota are prosobranchs, only 2% of the Oahu land snails are. However, the remaining pulmonates are still significantly ($G = 30.5$; $p << 0.001$) more often single island endemics on Oahu (87%, $N = 278$) than on Rota (25%, $N = 16$).

Further, all but three of the Rota pumonate families (*i. e.*, Achatinellidae, Succineidae, Endodontoidea, and Helicarionidae) belong to groups that are well represented on Oahu. Thus it appears that phylogenetic bias is not the cause of the differences. Intra-island speciation, which generates large numbers of endemics *in situ* has progressed to large radiations in several lineages on Oahu, but less so on Rota (but see below), and this process is expected to increase endemicity on Oahu. Finally the Mariana Islands lie in the western Pacific typhoon trough and are frequently battered by these catastrophic storms. Wind dispersal and transport by birds and possibly bats are believed to be perhaps the most important agents of dispersal among Pacific island land snails (Rees, 1965; Kondo, 1970; Vagvolgyi, 1975). The much greater availability of wind transport provides a site-specific explanation, and also matches observations in the marine environment, where storms have been shown to exert a major control on the distribution of marine organisms in the area (Kerr *et al.*, 1993; Kerr, 1994).

Assimineids are the most diverse family of land snails on both Guam and Rota. The Mariana Islands are the only oceanic Pacific island group with a diverse assemblage of assimineids. The Philippine Islands also support a diverse assemblage of assimineids but are continental islands. Of the 19 assimineid species recognized on Rota at this time; nine may be endemic, although six of the possible endemics are known from fewer than 20 specimens at three sites, making their identification and range among the islands tentative.

It appears that speciation within the Mariana Islands has occurred at least in the families Assimineidae, Charopidae, and Partulidae. There is clear evidence for both inter- and intra-island speciation within the Assimineidae and Charopidae. The *Omphalotropis* "carinate" species group forms a well-defined portion of the Rota assimineid fauna, with no apparent close relatives known from any of the other Mariana Islands. A single ancestral colonist is hypothesized for the radiation of the five nominal species in this complex. The frequent multi-island ranges of species in the families Assimineidae and Partulidae indicate that inter-island speciation could have been the main form of diversification in their evolutionary history. This is true as well for the Charopidae among the Mariana Islands, even though endodontoid land snails are renowned for their almost strict single-island endemicity (Solem, 1983).

Habitat destruction and introduced predators appear to have caused the decline and even possible extirpation of all species in three families of pulmonate land snails (Charopidae, Succineidae, and Pupillidae) on Rota since 1949. *Pythia scarabaeus*, a member of the Ellobiidae, could also be extirpated. In addition to the invertebrate predators, introduced feral mammals (pigs and rats) could have contributed to the decline of some species. The species in two other pulmonate families (Partulidae, Achatinellidae) have drastically declined since 1949. Partulids were abundant enough on Rota in 1969 for Eldredge (1969: 27) to state "these [partulids] are common enough to be collected for ornamentation and jewelry." In contrast, only five *Partula* populations are known to remain on Rota today.

Thirty-five of the 46 taxa known from Rota were encountered in the material from paleontological excavations at As Matmos and Payapai caves. These fossil assemblages thus represent a remarkably complete record of the island's malacofauna. The absence of many species in the material collected from the caves is readily explained: *Subulina octona*, *Achatina fulica*, and *Gonaxis kibwziensis* were introduced to the island in historic times; *Samoana fragilis* was only known from a single site on the island and has a thin fragile shell; the other four species are assimineids of which some seem to have naturally small ranges on Rota (*Omphalotropis octhogyra*, *O. quadrasi*) or are restricted to certain habitats (*O.* cf. *submaritima*, *O.* sp. 7). The number of land snail species recorded from these cave sites is high compared to other studies on land snail remains in archaeological settings (Christensen and Kirch, 1981; Chambers and Steadman, 1986). Paleontological excavations at As Matmos and Payapai caves reveal 13 taxa which no longer occur on the island, 11 of which cannot be matched to any species recorded previously from Guam or Rota. An abundance of *Lamellidea* shells in the paleontological excavations indicates they were much more common in the past at these two sites than they are today.

Of the 43 taxa found on Rota during 1994, 1995, and 1996 only 9% are considered surviving well, while 23% are surviving with questionable status. All families of land snails except Diplommatinidae have component species which are declining. Twelve percent of the species are potentially extirpated, while 56% of the fauna is considered to have declined. In conclusion, all evidence gathered to date suggests declines in populations, ranges, and species numbers of many land snails on Rota.

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LITERATURE CITED

Abbott, T. R. 1949. New syncerid mollusks from the Mariana Islands (Gastropoda, Prosobranchiata, Synceridae). *Occasional Papers of the Bernice P. Bishop Museum* 19(15):261-274.

Baker, H. B. 1938. Zonitid snails from Pacific islands part 1. Southern genera of Microcystinae. *Bernice P. Bishop Museum Bulletin* 158:1-102, pls. 1-20.

Baker, H. B. 1940. Zonitid snails from Pacific islands part 2. Hawaiian genera of Microcystinae. *Bernice P. Bishop Museum Bulletin* 165:103-201, pls. 21-42.

Balouet, J. C. and S. L. Olson. 1989. Fossil birds from Late Quaternary deposits in New Caledonia. *Smithsonian Contributions to Zoology* 469:1-38.

Chamberlin, J. L. 1952. *Final Report on an Ecology and Population Study of the Giant African Snail on Tinian, Mariana Islands.* Mimeographed unpublished report, Pacific Science Board, Honolulu. 30 pp.

Chambers, S. M. and D. W. Steadman. 1986. Holocene terrestrial gastropod faunas from Isla Santa Cruz and Isla Floreana, Galápagos: evidence for late Holocene declines. *Transactions of the San Diego Society of Natural History* 21(6):89-110.

Christensen, C. C. and P. V. Kirch. 1981. Nonmarine mollusks from archaeological sites on Tikopia, southeastern Solomon Islands. *Pacific Science* 35(1):75-88.

Clarke, B., J. Murray, and M. S. Johnson. 1984. The extinction of endemic species by a program of biological control. *Pacific Science* 38(2):97-104.

Cooke, C. M., Jr., and Y. Kondo. 1961. Revision of Tornatellinidae and Achatinellidae (Gastropoda, Pulmonata). *Bernice P. Bishop Museum Bulletin* 221(1960):1-303.

Cowie, R. H. 1992. Evolution and extinction of Partulidae, endemic Pacific island land snails. *Philosophical Transactions of the Royal Society of London* 335B:167-191.

Cowie, R. H. 1996. Variation in species diversity and shell shape in Hawaiian land snails: in situ speciation and ecological relationships. *Evolution* 49(6):1191-1202.

Cowie, R. H., N. L. Evenhuis, and C. C. Christensen. 1995. *Catalog of the Native Land and Freshwater Molluscs of the Hawaiian Islands.* Backhuys Publishers, Leiden. 248 pp.

Crampton, H. E. 1925. Studies on the variation, distribution, and evolution of the genus *Partula*. Carnegie Institute Washington, Publication 228A:1-116.

Eldredge, L. G. 1969. Notes on some terrestrial and freshwater invertebrates. *In: Biological Results of an Expedition to Rota, Mariana Islands,* R. T. Tsuda, ed. p. 27. Division of Biosciences and Marine Studies, University of Guam Miscellaneous Report 4, Agana.

Eldredge, L. G. and B. D. Smith. 1994. Introductions and transfers of the triclad flatworm *Platydemus manokwari*. *Tentacle* 3:8.

Hadfield, M. G. 1986. Extinction in Hawaiian achatinelline snails. *Malacologia* 27(1):67-81.

Hadfield, M. G., S. E. Miller, and A. H. Carwile. 1993. The decimation of endemic Hawai'ian tree snails by alien predators. *American Zoologist* 33:610-622.

Hadfield, M. G. and B. S. Mountain. 1980. A field study of a vanishing species, *Achatinella mustelina* (Gastropoda, Pulmonata), in the Waianae mountains of Oahu. *Pacific Science* 34(4):345-358.

Hopper, D. R. and B. D. Smith. 1992. Status of tree snails (Gastropoda: Partulidae) on Guam, with a resurvey of sites studied by H. E. Crampton in 1920. *Pacific Science* 46(1):77-85.

Kerr, A. M. 1994. Effects of typhoon-generated waves on windward and leeward assemblages of holothuroids. *Proceedings of the Seventh International Coral Reef Symposium, Guam* 1:308.

Kerr, A. M., E. M. Stoffel, and R. L. Yoon. 1993. Abundance distribution of holothuroids (Echinodermata: Holothuroidea) on a windward and leeward fringing coral reef, Guam, Mariana Islands. *Bulletin of Marine Science* 52(2):780-791.

Kondo, Y. 1952. *Report on Carnivorous Snail Experiment on Agiguan Island; Primary and Secondary* Achatina*-free Areas on Rota and Gigantism among* Achatina *on Guam.* Mimeographed unpublished report, Pacific Science Board, Honolulu. 50 pp.

Kondo, Y. 1970. Some aspects of Mariana Islands Partulidae (Mollusca, Pulmonata). *Occasional Papers of Bernice P. Bishop Museum* 24(5):73-90.

Kurozumi, T. 1994. Land molluscs from the northern Mariana Islands, Micronesia. *Natural History Research, Natural History Museum and Institute, Chiba, Special Issue* 1:133-119.

Lange, W. H., Jr. 1950. History and feeding habits of the giant African snail on Saipan. *Pacific Science* 4(4):323-35.

Mead, A. R. and Y. Kondo. 1950. *Part II. The Problem of the Giant African Snail* (Achatina fulica) *in Palau, Pagan and Guam* (by Kondo). Mimeographed unpublished report, Pacific Science Board, Honolulu. 43 pp.

Moellendorff, O. F. von. 1900. The land shells of the Caroline Islands. *The Journal of Malacology* 7(5):101-126.

Muniappan, R. 1983. Biological control of the giant African snail. *Alafua Agricultural Bulletin* 8(1):43-46.

Murray, J., E. Murray, M. S. Johnson, and B. Clarke. 1988. The extinction of *Partula* on Moorea. *Pacific Science* 42(3-4):150-153.

Paulay, G. 1994. Biodiversity on oceanic islands: its origin and extinction. *American Zoologist* 34:134-144.

Preece, R. C. 1995. Systematic review of the land snails of the Pitcairn Islands. *Biological Journal of the Linnean Society* 56:273-307.

Quadras, J. F. and O. F. von Moellendorff. 1894a. Diagnoses specierum novarum a J. F. Quadras in insulis Mariannis collectarum scripserunt. *Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft* 26(1/2):13-22.

Quadras, J. F. and O. F. von Moellendorff. 1894b. Diagnoses specierum novarum a J. F. Quadras in insulis Mariannis collectarum scripserunt. *Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft* 26(3/4):33-42.

Ress, W. J. 1965. The aerial dispersal of Mollusca. *Proceedings of the Malacological Society of London* 35:269-282.

Smith, B. 1993 (September 15). *Working List of the Terrestrial Gastropods of Guam.* Richard "E." Dickinson Memorial Mollusc Collection, Marine Laboratory, University of Guam, Mangilao. 7 pp.

Solem, A. 1959. Systematics and zoogeography of the land and freshwater Mollusca of the New Hebrides. *Fieldiana: Zoology* 43:1-359, pls. 1-34.

Solem, A. 1976. *Endodontoid Land Snails from Pacific Islands (Mollusca: Pulmonata: Sigmurethra). Part I. Family Endodontidae.* Field Museum of Natural History, Chicago. 508 pp.

Solem, A. 1983. *Endodontoid Land Snails from Pacific Islands (Mollusca: Pulmonata: Sigmurethra). Part I. Families Punctidae and Charopidae, Zoogeography.* Field Museum of Natural History, Chicago. 336 pp.

Solem, A. 1988. Non-camaenid land snails of the Kimberley and Northern Territory, Australia. I. Systematics, affinities and ranges. *Invertebrate Taxonomy* 2(4):455-604.

Solem, A. 1990. How many Hawaiian land snail species are left? and what can we do for them. *Bishop Museum Occasional Papers* 30:27-40.

Steadman, D. W. 1992. Extinct and extirpated birds from Rota, Mariana Islands. *Micronesica* 25(1):71-84.

Steadman, D. W. 1993. Biogeography of Tongan birds before and after human impact. *Proceedings of the National Academy of Sciences* 90:818-822.

Thiele, J. 1929. *Handbook of Systematic Malacology, Part 1: Loricata; Gastropoda: Prosobranchia.* (1992 English translation of the original German by R. Bieler and P. M. Mikkelsen, eds). Smithsonian Institution Libraries and National Science Foundation, Washington D.C. 625 pp.

Thompson, F. G. and S. P. Dance. 1983. Non-marine mollusks of Borneo, II Pulmonata: Pupillidae, Clausiliidae, III Prosobranchia: Hydrocenidae, Helicinidae. *Bulletin of the Florida State Museum* 29(3):102-152.

Thompson, F. G. and E. L. Huck. 1985. The land snail family Hydrocenidae in Vanuatu (New Hebrides Islands), and comments on other Pacific Island species. *The Nautilus* 99(2-3):81-84.

Vagvolgyi, J. 1975. Body size, aerial dispersal, and origin of the Pacific land snail fauna. *Systematic Zoology* 24(4):465-488.

Zilch, A. 1953. Die Typen und Typoide des Natur-Museums Senckenberg, 9): Mollusca, Cyclophoridae, Diplommatininae. *Archiv für Molluskenkunde* 82(1/3):1-47.

Zilch, A. 1962. Die Typen und Typoide des Natur-Museums Senckenberg, 26): Mollusca, Achatinellacea. *Archiv für Molluskenkunde* 91(1/3):77-94.

Zilch, A. 1967. Die Typen und Typoide des Natur-Museums Senckenberg, 36): Mollusca, Assimineidae. *Archiv für Molluskenkunde* 96(1/2):67-100.

Zilch, A. 1973a. Die Typen und Typoide des Natur-Museums Senckenberg, 52): Mollusca: Hydrocenidae. *Archiv für Molluskenkunde* 103(4/6):263-272.

Zilch, A. 1973b. Die Typen und Typoide des Natur-Museums Senckenberg, 53): Mollusca: Truncatellida. *Archiv für Molluskenkunde* 103(4/6):273-282.

Zilch, A. 1978. Die Typen und Typoide des Natur-Museums Senckenberg, 60): Mollusca: Succineacea. *Archiv für Molluskenkunde* 109(1/3):109-136.

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Biogeography of the genera of Naticidae (Gastropoda) in the Indo-Pacific

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Abstract: The biogeographic distribution of genera of the family Naticidae (Mollusca: Gastropoda) in the tropical Indo-Pacific is delineated and follows a pattern found in other marine molluscan families. Within the monophyletic clades "Naticinae" and "Sininae" the greatest Indo-Pacific species-level diversity is found in the western Indian Ocean and in Australia; with a marked eastward decline in biodiversity from Melanesia and Micronesia to Polynesia and Hawaii. At the generic level, the broad geographic distribution and lack of endemicity precludes reconstruction of the vicariant history of these genera. The present-day distribution of species and genera is largely a function of larval dispersal, not of vicariant events.

The two questions for a biogeographical analysis are (1) "what are the patterns?" [descriptive biogeography] and (2) "how did the patterns come about?" [analytical or phylogenetic biogeography].

DESCRIPTIVE BIOGEOGRAPHY

The Naticidae is a cosmopolitan family of marine prosobranch gastropods, found burrowing in sandy habitats, usually in shallow, nearshore waters. There are about 260-270 Recent species in the family, which originated in the Triassic. The greatest species and generic diversity is in tropical regions, and the analyses here are based upon tropical taxa, with emphasis on the Indo-Pacific biogeographic region.

The phylogeny of the Naticidae (Kabat, unpubl.), although reasonably well-resolved for the Recent genera alone, does have several areas that require more research in order to resolve polytomies or less well-defined clades. In traditional classifications, four subfamilies have been recognized (*e. g.* Marincovich, 1977). One, the "Ampullospirinae" is actually a grade not a clade; as it is primarily found in Arctic and Antarctic regions, it is not further considered here. The second traditional subfamily, the "Polinicinae," is also a grade and the relationships of its genera remain less well-resolved. Although the "Polinicinae" includes several tropical genera, it was not analyzed in this study.

The remaining two traditional subfamilies do form monophyletic, well-defined clades, and are the focus of this study. The "Naticinae" has 15 genera (three now extinct), of which ten are found in Recent tropical regions. The "Sininae" has five genera, all restricted to temperate-tropical regions.

First, consider the smaller Sininae. Fig. 1 shows the cladistic relationships of the five genera. Because the Sininae forms a monophyletic clade within the "Polinicinae" grade, its actual ranking (as a subfamily or tribe) is debatable. However, the "Sininae" does represent a monophyletic clade which is essential for this biogeographic analysis.

There are 50 Recent species of the Sininae (Table 1), among which 33 are found in the Indo-Pacific. The greatest specific and generic diversity is found in the Indo-Pacific, with a declining eastward gradient from the eastern Pacific to the western Atlantic and the least biodiversity in the eastern Atlantic. *Haliotinella* is an especially rare and cryptic naticid genus, whose three species are known from fewer than 20 specimens; its supposed absence in the eastern Pacific or even the eastern Atlantic may be a collecting artifact. When this geographic distribution is mapped onto the cladogram (Fig. 1), no apparent correlation with the position on the cladogram of the various genera is shown.

Now, to consider the more speciose subfamily Naticinae. Fig. 2 shows the cladistic relationships of the 12 Recent genera of this subfamily. However, two naticine genera are not found in tropical regions, and thus were not analyzed for the tropical species-level diversity in this study.

The species diversity of the ten genera of tropical Naticinae (Table 2) shows the highest specific diversity is found in the Indo-Pacific. In contrast to the Sininae, the Naticinae do not show a declining gradient from the eastern Pacific eastward to the eastern Atlantic; in fact, the gradient

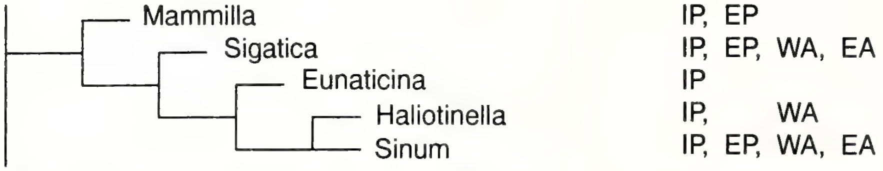

Fig. 1. Cladogram of the "Sininae" with the geographic distribution indicated for each genus. EA, eastern Atlantic; EP, eastern Pacific; IP, Indo-Pacific; WA, western Atlantic.

runs in the opposite, westward direction. There are two amphi-Atlantic species in this subfamily (along with two others in the genus *Polinices*); the eastern Pacific does not share any naticid species with the Indo-Pacific. In other words, the only trans-regional tropical naticid species are the four amphi-Atlantic species.

At the generic level, there is no gradient in generic diversity of the Naticinae in these regions, with six genera in the Indo-Pacific and eastern Pacific, seven in the western Atlantic, and five in the eastern Atlantic. Only two genera are truly endemic to one region: *Tanea* in the Indo-Pacific and *Cochlis* in the tropical eastern Atlantic. *Lunaia*, known from one species in the eastern Pacific, is a poorly defined genus and is potentially a synonym of another genus. *Carinacca*, which has one Recent species in the western Atlantic, was originally described as a fossil taxon from New Zealand. The geographic distribution of these genera is mapped onto the cladogram (Fig. 2); as with the "Sininae," there is no obvious pattern in the generic distribution in relation to the generic phylogeny.

The last component of the descriptive phase of this study comprises a species-level analysis of the tropical Indo-Pacific Naticinae. I have determined the distribution of the known species of this fauna, based primarily on examination of numerous museum records, and to a lesser extent on reliable literature records. Although the data are broken down by country and island group, for convenience they are here combined into ten broad regions within the Indo-Pacific: western Indian Ocean [Africa to India and Sri Lanka]; southeast Asia [Burma to China, Indonesia, Philippines]; Japan (including the Ryukyus); Australia and New Zealand; western Melanesia [New Guinea and Solomon Islands]; eastern Melanesia [Vanuatu, New Caledonia, Fiji, Wallis and Futuna]; Micronesia [Marianas, Palau, Carolines, Marshall, western Kiribati]; western Polynesia [Tonga, Niue, Samoa, Tokelau, Phoenix]; eastern Polynesia [Cook and Line Islands, French Polynesia]; and Hawaii.

There are six genera of Naticinae in the Indo-Pacific fauna, comprising at least 52 species. Several rare species described from tropical Japan were not included here, as I have not seen any material, and the descriptions were not sufficiently detailed for me to determine whether these taxa were valid or junior synonyms.

The greatest species-level diversity is found in the western Indian Ocean and in Australia, with slightly lower numbers in southeast Asia. There is a decided eastward reduction in species diversity, from western Melanesia to eastern Melanesia and Micronesia, and even more so into Polynesia and Hawaii (Table 3). Note that these comparisons of regional diversity are *not* based on regions of comparable size, either overall or in suitable habitat area.

Of these 52 species, exactly half (26) are endemic to one of these broadly-defined regions. Most endemic

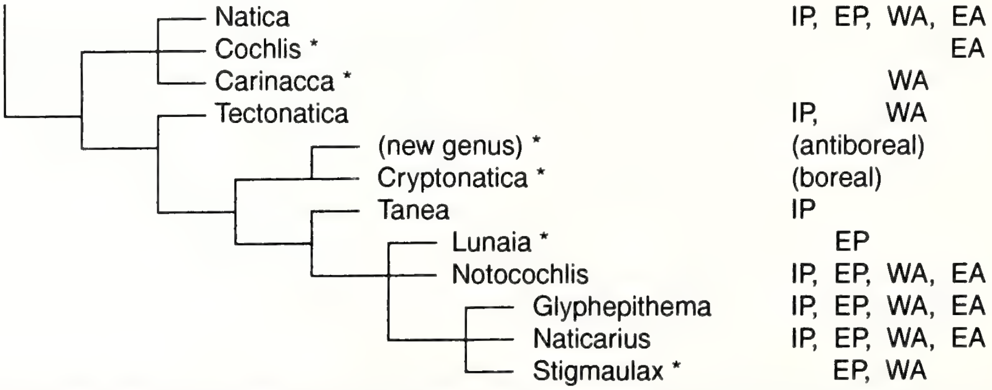

Fig. 2. Cladogram of the Recent genera of "Naticinae" with the geographic distribution indicated for each genus. EA, eastern Atlantic; EP, eastern Pacific; IP, Indo-Pacific; WA, western Atlantic; *, Recent genera *not* found in Indo-Pacific.

Table 1. "Sininae" (Recent), species diversity.

	Indo-Pacific	E. Pacific	W. Atlantic	E. Atlantic
Mammilla	9	1	–	–
Sigatica	6	1	2	1
Eunaticina	6	–	–	–
Sinum	10	6	3	2
Haliotinella	2	–	1	–
TOTAL	33	8	6	3

species are found in either the western Indian Ocean or in Australia (both tropical and warm-temperate); relatively few in the central Pacific proper. Most of the western Indian Ocean endemics are actually restricted to a quite smaller area within this region, such as the Persian Gulf or Mozambique and Natal. An alternative approach to analyzing endemicity would be to start with corresponding species ranges and to define areas based on that rather than the "bottom down" approach used herein.

An analysis of the species found in more than one of these regions indicates that eight (15%) are found in two to four regions, nine (17%) in five to seven regions, and nine (17%) in eight to ten regions, including three being found in all ten regions.

A more detailed breakdown of species-level diversity, by genus, is shown in Table 4. The genera are listed in order of their appearance on the cladogram, with the oldest genus first, and the most derived last. For the five genera containing more than one Indo-Pacific species, there exists a west to east gradient in species number and in endemicity. Table 5 lists the species and their distribution for each genus; the numbers in parentheses indicate species endemic to one region.

There are five species of Naticinae in Hawaii but only one (20%) is endemic. Although based on a small sample, this does agree with the results of Kay and Palumbi (1987) who found that of the 234 species of "Mesogastropoda" found in Hawaii, 49 (21%) were endemic to Hawaii. A nearly similar percentage (18%) of the "Neogastropoda" are also endemic to Hawaii; about twice as many "Archaeogastropoda" (39%) and Bivalvia (51%) species in Hawaii are endemics, but no explanation for this disparity among molluscan groups was offered by Kay and Palumbi (1987). G. Paulay (in litt., 15 July 1995) suggested that the former was due to the lack of planktotrophic development in most "archaeogastropods" while the endemicity of the Bivalvia could be a taxonomic artifact of the monograph of Dall *et al.* (1938) which had numerous (over 130) supposedly new bivalve species endemic to Hawaii but many of which are actually known or probable synonyms of previously described species.

PHYLOGENETIC BIOGEOGRAPHY

With the descriptive data in hand, what are the possible explanations for the observed patterns? More importantly, what are the caveats that should be noted, or pitfalls likely to arise in such analyses?

1. At the species level, both groups analyzed have their highest biodiversity in the Indo-Pacific. The contrasting gradients in the four oceanic regions – eastward decline in the Sininae and westward decline in the Naticinae – do not suggest any simple pattern. It might be thought that these two clades had opposite "tracks" or biogeographic paths in their evolutionary history. A track analysis must be based on the actual taxonomic units that cross the boundaries between oceanic regions, in this case genera instead of subfamilies. One would need a phylogeny of species within a single genus to better address this problem.

2. At the generic level, the results are less conclusive. The eastward decline in generic diversity in the Sininae matches the similar decline in its species diversity. However, for the Naticinae, the generic diversity of all four oceanic regions is comparable (five to seven genera per region) and does not match the gradient of the species-level diversity. One also needs to know the relative ages of these two subfamilies: if the Naticinae is older, then there has been sufficient time for most of the genera to spread to all tropical regions.

More importantly, mapping the geographic areas onto the generic cladograms did not show any patterns as might have been predicted by theories of either traditional biogeography, or of cladistic (vicariance) biogeography.

To briefly review vicariance biogeography (Wiley, 1988), the keystone concept is that of vicariant events: geographic separations which serve to separate populations of one taxon leading to speciation, are shown by the geographic distribution of sister taxa. Vicariant phenomena can be extended to the analysis of higher taxa, such as genera or

Table 2. Tropical "Naticinae" (Recent), species diversity.

	Indo-Pacific	E. Pacific	W. Atlantic	E. Atlantic
Natica	13	2	1	5
Cochlis	–	–	–	2
Carinacca	–	–	1	–
Tectonatica	8	–	1	–
Tanea	13	–	–	–
Lunaia	–	1	–	–
Notocochlis	7	5	6 *	6 *
Glyphepithema	1	1	2	1
Naticarius	10	2	1	3
Stigmaulax	–	2	2	–
TOTAL	52	13	14 *	17 *

* includes two amphi-Atlantic species.

Table 3. Generic and species diversity of Indo-Pacific "Naticinae" by region.

Region	# genera	# species	# endemic species
W. Indian Ocean	6	30	10
Southeast Asia	6	27	4
Japan	5	17	3
Australia & N. Z.	6	31	7
W. Melanesia	6	21	0
E. Melanesia	6	18	1
Micronesia	6	14	0
W. Polynesia	5	8	0
E. Polynesia	4	7	0
Hawaii	4	5	1
TOTAL	6	52	26

families. In contrast, traditional biogeography relies on the concept of centers of origin and on dispersal as the primary mechanism for the origin of disjunct populations.

Perhaps the most important consideration is that these theories of vicariance biogeography were largely based upon the study of non-marine organisms, particularly those with very limited dispersal ability. In such groups the present-day distribution can and often does reflect the phylogenetic history of the taxa. In contrast, most marine invertebrates have significant dispersal capabilities, typically through their planktonic larval stages. What makes such analyses difficult for marine taxa is that while a vicariant event may well have led to speciation, or other cladogenetic events, the subsequent dispersal of populations across the original geographic barrier will in all probability conceal this separation (Gosliner, 1994). Hence the present-day distribution of sister taxa does not allow reconstruction of the vicariant events.

An alternative hypothesis for speciation in the marine realm is that of founder effects, where there is chance dispersal of a species beyond its normal range with subsequent isolation of the newly founded population. Kay (1984: 25-26) suggested that such peripheral origins could explain the distribution of certain Indo-Pacific species. Some species thus subsequently disperse from their isolated location back (westward) to the range of their sister species which therefore becomes the center of highest diversity but *not* the "center of origin" (see also Ladd, 1960: 140-141; contrast Ekman, 1953: 18 ff.).

The second consideration is that a proper analysis of vicariance biogeography must have at least one endemic taxon in each geographic region (Harold and Mooi, 1994). If the regions are sufficiently large enough, then this criterion is more easily met. Again, this criterion is *not* met by my study here, or indeed several other biogeographic analyses of marine invertebrates (such as Wallace *et al.*, 1991).

At the generic level, there is not at least one naticid genus endemic to each of the four oceanic regions, as most genera are pan-tropical or else found in two to three oceanic regions. The few truly endemic genera are not sufficient to do a proper vicariance analysis. Within the four oceanic regions, most of these genera are broadly distributed across each region, so that subdividing the four oceanic regions into smaller biogeographic provinces still would not permit such an analysis within each region.

At the species level, again similar problems arise, with an additional complication. I do not have a phylogenetic analysis of species within a genus, and the traditional morphological characters as used in the generic analysis are not suitable for a species analysis (because I tried to use only characters that varied between genera, *not* among species within a genus). The traditional characters by which naticid species can be readily differentiated do not form useful transformation series, thus rendering difficult a phylogenetic analysis of the species.

But let us suppose that there was a molecular analysis of the species for several naticid genera, resulting in a reasonably well-resolved cladogram. In that case, could an analysis of vicariance biogeography be conducted? The two caveats mentioned with the genera apply with equal force at the species level: (1) regardless of however many or few regions are used, there must be at least one species endemic to each region; and (2) the subsequent larval dispersal is likely to have concealed the original vicariant disjunctions.

Recall that the patterns of species endemicity in the subfamily Naticinae were decidedly non-random: the bulk

Table 4. Species-level diversity for genera of Indo-Pacific "Naticinae." Numbers in parentheses are species endemic to one region. ANZ, Australia and New Zealand; EMel, eastern Melansia; EPol, eastern Polynesia; Haw, Hawaii; J, Japan; Mic, Micronesia; SeA, southeast Asia; WIO, western Indian Ocean; WMel, western Melanesia; WPol, western Polynesia.

	#	(# end.)	WIO	SeA	J	ANZ	WMel	EMel	Mic	WPol	EPol	Haw
Natica	13	(7)	12(6)	7(1)	4	6	5	4	3	0	0	0
Tectonatica	8	(4)	6(2)	4(10	0	5(1)	4	4	4	3	3	1
Tanea	13	(6)	5(1)	6	5(1)	10(3)	5	3	1	1	0	1(1)
Notocochlis	7	(4)	3(1)	4(1)	3	4(2)	2	2	2	2	2	2
Naticarius	10	(5)	3	5(1)	4(2)	5(1)	4	4(1)	3	1	2	0
Glypheptithema	1	(0)	1	1	1	1	1	1	1	1	1	1

Table 5. Species distributions for the tropical Indo-Pacific Naticinae [geographic abbreviations as in Table 4]. An asterisk * indicates species endemic to one region.

	WIO	SeA	J	ANZ	WMel	EMel	Mic	WPol	EPol	Haw
Glyphepithema										
alapapilionis (Röding, 1798)	x	x	x	x	x	x	x	x	x	x
Natica										
arachnoidea (Gmelin, 1791)	x	x	0	x	x	x	x	0	0	0
buriasensis Récluz, 1843	x	x	x	x	x	x	x	0	0	0
fasciata (Röding, 1798)	x	x	0	x	x	x	x	0	0	0
**forskalii* Sowerby, 1825	x	0	0	0	0	0	0	0	0	0
**ponsonbyi* Melvill, 1899	x	0	0	0	0	0	0	0	0	0
**pulicaris* Philippi, 1852	x	0	0	0	0	0	0	0	0	0
**pygmaea* Philippi, 1842	x	0	0	0	0	0	0	0	0	0
**queketti* Sowerby, 1894	x	0	0	0	0	0	0	0	0	0
**schepmani* Thiele, 1925	0	x	0	0	0	0	0	0	0	0
**scutulata* Philippi, 1852	x	0	0	0	0	0	0	0	0	0
stellata Hedley, 1913	x	x	x	x	x	0	0	0	0	0
tigrina (Röding, 1798)	x	x	x	x	0	0	0	0	0	0
vitellus (Linné, 1758)	x	x	x	x	x	x	0	0	0	0
Naticarius										
**colliei* (Récluz, 1844)	0	0	0	x	0	0	0	0	0	0
**concinna* (Dunker, 1860)	0	0	x	0	0	0	0	0	0	0
**excellens* (Azuma, 1961)	0	0	x	0	0	0	0	0	0	0
insecta (Jousseaume, 1874)	0	0	0	x	x	0	0	0	0	0
**lineozona* (Jousseaume, 1874)	0	0	0	0	0	x	0	0	0	0
manceli (Jousseaume, 1874)	x	x	0	0	0	0	0	0	0	0
onca (Röding, 1798)	x	x	x	x	x	x	x	x	0	0
orientalis (Gmelin, 1791)	x	x	x	x	x	x	x	0	x	0
**philippinensis* (Watson, 1881)	0	x	0	0	0	0	0	0	0	0
zonalis (Récluz, 1850)	0	x	0	x	x	x	0	0	x	0
Notocochlis										
cernica (Jousseaume, 1874)	x	x	x	x	x	x	x	x	x	x
gualtieriana (Récluz, 1844)	x	x	x	x	x	x	x	x	x	x
**insularis* (Watson, 1886)	0	x	0	0	0	0	0	0	0	0
nipponensis (Kuroda, 1961)	0	x	x	0	0	0	0	0	0	0
**subcostata* (Tenison-Woods, 1876)	0	0	0	x	0	0	0	0	0	0
**tranquilla* (Melvill and Standen, 1901)	x	0	0	0	0	0	0	0	0	0
**zonulata* (Thiele, 1930)	0	0	0	x	0	0	0	0	0	0
Tanea										
areolata (Récluz, 1844)	x	x	x	x	x	x	x	x	0	0
euzona (Récluz, 1844)	x	x	0	x	x	x	0	0	0	0
**hilaris* (Sowerby, 1914)	0	0	x	0	0	0	0	0	0	0
lineata (Röding, 1798)	x	x	x	x	x	0	0	0	0	0
**luculenta* (Iredale, 1929)	0	0	0	x	0	0	0	0	0	0
mozaica (Sowerby, 1883)	0	0	0	x	x	x	0	0	0	0
picta (Récluz, 1844)	x	x	0	x	0	0	0	0	0	0
**sagittata* (Menke, 1843)	0	0	0	x	0	0	0	0	0	0
tabularis (Kuroda, 1961)	0	x	x	x	0	0	0	0	0	0
undulata (Röding, 1798)	0	x	x	x	x	0	0	0	0	0
**zelandica* (Quoy and Gaimard, 1832)	0	0	0	x	0	0	0	0	0	0
*new sp. 1	x	0	0	0	0	0	0	0	0	0
*new sp. 2	0	0	0	0	0	0	0	0	0	x
Tectonatica										
bougei (Sowerby, 1908)	x	0	0	x	x	x	x	x	x	x
robillardi (Sowerby, 1894)	x	x	0	x	x	x	x	x	x	0
**simplex* (Sowerby, 1897)	x	0	0	0	0	0	0	0	0	0
**shorehami* (Pritchard and Gatliff, 1900)	0	0	0	x	0	0	0	0	0	0
suffusa (Reeve, 1855)	x	x	0	x	x	x	x	0	0	0
**tecta* (Anton, 1838)	x	0	0	0	0	0	0	0	0	0
violacea (Sowerby, 1825)	x	x	0	x	x	x	x	x	x	0
*new sp. 1	0	x	0	0	0	0	0	0	0	0

of endemicity was in the western Indian Ocean and Australia, with significantly reduced endemicity elsewhere in the Indo-Pacific (but note the caveat *re* defining the regions). Although there are several schemes for subdividing the Indo-Pacific into biogeographic provinces (*e. g.* Kay, 1980; Dahl, 1984; Blum, 1989; Stoddart, 1992), none would allow us to have at least one endemic naticine species in each province. Even some very broadly defined regions, such as western and eastern Polynesia, do not have any endemic naticine species.

The "bugaboo" of larval dispersal is, in my opinion, the greatest barrier to biogeographic analyses of marine invertebrates. This intellectual barrier is probably even more important than the geographical barriers caused by the elevation of the Panama land bridge, or the closure of the Suez between the Mediterranean and the Red Sea, in reconstructing the biogeographic history of marine invertebrates!

We are all familiar, thanks to the studies of Scheltema and others presented in this symposium (*e.g.* Kohn, Bieler), of the remarkable abilities of the larval stages of marine invertebrates not only to cover vast distances, but also to maintain the genetic integrity of species across an oceanic region. It should come as no surprise to biogeographers that such dispersal can and often will make it impossible for us to delineate the vicariant events based upon a study of the current geographic distribution of taxa, as Gosliner (1994) has noted.

For the family Naticidae, there is little or no adult dispersal of biogeographic relevance. However, the majority of tropical species whose development is known or can be inferred from protoconch size have planktonic larvae. Direct development, or the hatching of benthic juveniles, is documented primarily for cold-water species and I have predicted its occurrence for several tropical species with restricted ranges (endemic to a small region) based upon their large protoconch sizes. Some other endemic tropical naticid species may have lecithotrophic, or short-dispersing larvae, although this needs to be documented from study of the egg masses themselves. In contrast, most of the widespread tropical naticid species, including all four tropical amphi-Atlantic naticids, are known to have planktonic development, usually documented as planktotrophic (*e. g.* Thorson, 1940; Bandel, 1976).

If all naticids had direct development, or at least short-term lecithotrophic development, then their dispersal abilities would be significantly restricted, and it would be easier to reconstruct the vicariant history of this group. However, the admixture of all three modes of larval development within a single genus would result in dispersal patterns confounding the original vicariant patterns. Again, it must be emphasized that vicariant theory was based primarily on non-marine organisms with limited dispersal capability. I seriously question whether we can apply cladistic biogeography to marine invertebrates, at least to those groups with high larval dispersal ability or those that are readily dispersed by rafting.

There is one study on marine organisms, the fish family Chaetodontidae (Blum, 1989) for which satisfactory vicariant analyses could be conducted, as not only were the species-level relationships reasonably well known, but also there were sufficient numbers of endemic species among the regions. Nonetheless, most of the "barriers" which Blum recognized (1989: fig. 11, table 2) were based on only one or (seldom) two to three pairs of sister taxa, which may not be statistically significant considering the large numbers of tropical species (over 110) in this family. Indeed, Blum (1989: 10) stated that "almost all of the sister groups ... are broadly sympatric ... Thus most of the geography associated with early chaetodontid evolution has been obscured by subsequent dispersal." These problems will recur with other marine taxa. It might seem that one should avoid biogeographic conclusions based upon a small and carefully selected subset of a group, yet such a group (containing allopatric species) may allow reconstruction of the vicariant history prior to subsequent dispersal.

McMillan and Palumbi (1995) recently performed a molecular analysis on two of Blum's species groups in the Chaetodontidae. Their results from these two species groups, carefully chosen to include only allopatric species, showed fairly recent speciation events potentially attributable to Pleistocene glacio-eustatic sea level changes (see also Paulay, 1991).

I now briefly discuss whether the Naticidae fits into Springer's 1982 model of "Pacific Plate Biogeography" which was based primarily upon an analysis of the distribution of shorefishes, but also drew upon several invertebrate groups. Springer (1982) claimed that the margin of the Pacific plate represented a significant dispersal barrier, and thus a source of endemic species. For the Naticidae, at least, these results are not confirmed. Most widely-distributed species have dispersed right across the western (Asian) margin of the Pacific plate, as might be expected from the prevailing oceanic currents which naturally bear little relation to the distribution of tectonic plates on the ocean floor itself. Furthermore, most endemic naticid species are found neither within the Pacific plate itself, nor on its margin, but rather in the western Indian Ocean, or along the Australian continental shelf, both areas at some remove from the Pacific plate. Indeed, not a single species among the Naticinae is a "widespread Pacific plate endemic" and the sole species restricted to the Pacific plate is endemic to Hawaii.

In conclusion, this paper has demonstrated a number of biogeographic patterns based upon the descriptive biogeography of the genera and species of tropical Naticidae. However, explaining these patterns in a cladistic or vicariance context remains quite problematical, for several reasons which are equally applicable to most taxa of marine organisms. Although seemingly a "negative result" this does indicate the limited utility of such biogeographic theories, and suggests that an entirely different approach to reconstructing the geographic history of marine taxa is needed.

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LITERATURE CITED

Bandel, K. 1976. Die Gelege karibischer Vertreter aus den Überfamilien Strombacea, Naticacea und Tonnacea (Mesogastropoda) sowie Beobachtungen im Meer und Aquarium. *Mitteilungen aus dem Instituto Colombo-Alemán des Investigaciones Cientificas "Punta de Betin," Santa Marta* 8:105-139.

Blum, S. D. 1989. Biogeography of the Chaetodontidae: an analysis of allopatry among closely related species. *Environmental Biology of Fishes* 25(1-3):9-31.

Dahl, A. L. 1984. Biogeographical aspects of isolation in the Pacific. *Ambio* 13(5-6):302-305.

Dall, W. H., P. Bartsch, and H. A. Rehder. 1938. A manual of the Recent and fossil marine pelecypod mollusks of the Hawaiian Islands. *Bulletin of the Bernice P. Bishop Museum* 153:1-233, 58 pls.

Ekman, S. 1953. *Zoogeography of the Sea* (trans. E. Palmer). Sidgwick and Johnson, Ltd., London. xiv + 417 pp.

Gosliner, T. M. 1994. Biodiversity of tropical opisthobranch gastropod faunas. *Proceedings of the Seventh International Coral Reef Symposium, Guam* 2:702-709.

Harold, A. S. and R. D. Mooi. 1994. Areas of endemism: definition and recognition criteria. *Systematic Biology* 43(2):261-266.

Kay, E. A. 1980. Little worlds of the Pacific: an essay on Pacific Basin biogeography. *University of Hawaii, Harold L. Lyon Arboretum Lecture* 9:1-40.

Kay, E. A. 1984. Patterns of speciation in the Indo-West Pacific. *In: Biogeography of the Tropical Pacific*, F. J. Radovsky, P. H. Raven, and S. H. Sohmer, eds. pp. 15-31. Honolulu, Bishop Museum Special Publication 72.

Kay, E. A. and S. R. Palumbi. 1987. Endemism and evolution in Hawaiian marine invertebrates. *TREE [Trends in Ecology and Evolution]* 2(7):183-186.

Ladd, H. S. 1960. Origin of the Pacific island molluscan fauna. *American Journal of Science* 258A:137-150.

Marincovich, L. N. Jr. 1977. Cenozoic Naticidae (Mollusca: Gastropoda) of the northeastern Pacific. *Bulletins of American Paleontology* 70(294):165-494, pls. 17-42.

McMillan, W. O. and S. R. Palumbi. 1995. Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 260(1358):229-236.

Paulay, G. 1991 [1990]. Effects of late Cenozoic sea-level fluctuations on the bivalve faunas of tropical oceanic islands. *Paleobiology* 16(4):415-434.

Springer, V. G. 1982. Pacific plate biogeography, with special reference to shorefishes. *Smithsonian Contributions to Zoology* 367:iv + 182 pp. + errata.

Stoddart, D. R. 1992. Biogeography of the tropical Pacific. *Pacific Science* 46(2):276-293.

Thorson, G. [A. W.] 1940. Studies on the egg masses and larval development of Gastropoda from the Iranian Gulf. *Danish Scientific Investigations in Iran* 2:159-238.

Wallace, C. C., J. M. Pandolfi, A. Young, and J. Wolstenholme. 1991. Indo-Pacific coral biogeography: a case study from the *Acropora selago* group. *Australian Systematic Botany* 4:199-210.

Wiley, E. O. 1988. Vicariance biogeography. *Annual Review of Ecology and Systematics* 19:513-542.

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Indo-Pacific opisthobranch gastropod biogeography: how do we know what we don't know?

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Abstract: Recent studies on the biodiversity of Indo-Pacific opisthobranchs demonstrate that more than 3,400 species are known from the region, including more than 1,000 undescribed species. This estimate exceeds a recent estimate of worldwide opisthobranch diversity. Evidence from recent collections supports the contention that this figure represents a minimum diversity for the region and that many other undescribed species are presently undetected.

Distribution patterns of Indo-Pacific opisthobranchs remain poorly known. Recent rediscovery of previously described taxa from widely separated localities supports the idea that many fundamental distributional data are currently lacking. The apparent species composition of the Hawaiian opisthobranch fauna has increased by more than 75% in the last three years. This attests to the lack of baseline data even from localities that were believed to be well known.

Estimates of the percentage of the fauna endemic to a region range widely as is shown for the Hawaiian Islands. Despite this fact, it is apparent that the perceived level of endemism for Hawaiian opisthobranchs is decreasing with increasing taxonomic refinement and discovery of additional species. It is suggested that the disparity between levels of endemism of Hawaiian marine and terrestrial biotas is real rather than artifactual, as suggested by some previous workers.

Phylogenetic hypotheses, necessary for study of vicariant patterns in Indo-Pacific opisthobranchs, are limited in number. Of the phylogenies that do exist, none show vicariance of sister taxa within the Indo-Pacific, but exhibit marked sympatry between sister species. This suggests that subsequent to vicariance, dispersal has masked original allopatry of sister taxa. None of the opisthobranch taxa thus far studied have Pacific Plate endemics as described by Springer (1982) for shorefishes. However, it is clear that phylogenetic studies of opisthobranch taxa with more endemic representatives must be undertaken to further examine these patterns.

Studies of biodiversity of organisms are contingent upon reliable information regarding the species composition and distribution of taxa within and between different geographical regions. These distributional data are requisite for both classical and vicariant biogeographical studies. The tropical Indo-Pacific is unsurpassed in its species richness for most marine taxa and rivals tropical rainforests in its diversity. Owing to the vastness of the region, stretching from the eastern coast of Africa to the Hawaiian Islands, many localities remain poorly studied and and others imperfectly known. Winston (1988) estimated that 20-80% of the marine organisms inhabiting the region remain undescribed, depending upon the taxonomic group being considered. Few studies have focused upon acquiring more detailed estimates of the status of knowledge of marine taxa inhabiting the Indo-Pacific tropics or the potential impact of these data upon estimates of standing biodiversity. Ghiselin (1992) and Gosliner (1992) provided estimates of the status of knowledge regarding opisthobranch faunas at several localities within the Indo-Pacific tropics. Problems of comparability of data sets were noted.

Classical biogeographical studies have emphasized the composition of biotas from discrete geographic areas. The number of species known to occur in a particular area has been the primary focus of this approach. Determination of boundaries of biotic provinces has been another traditional concern of this descriptive approach. A third component has been the determination of biogeographic affinities to other geographical regions or provinces. The surrounding areas which share the largest number of species with the area of immediate concern are said to have the strongest biogeographic affinity to that area. Comparison of the percentage of species endemic to a region to those which are widespread provides an estimate of the isolation of that biota from other biotas either by means of strong barriers, limited dispersal, or both.

In contrast, vicariance biogeography is concerned with distributional comparisons of phylogenetically related taxa. In addition to taxonomic and distributional data, phylogenentic hypotheses are required to undertake comparisons of vicariant patterns of distribution. Vicariant patterns can only be recognized when there is geographical isolation of closely related taxa.

This paper critically evaluates the present status of knowledge of opisthobranch gastropods in the Indo-Pacific

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tropics. Several aspects of biodiversity and biogeography are explored, especially within the context of the kind of data that are required for studies of phylogenetics and vicariance biogeography.

METHODS

Several lines of investigation were pursued in order to survey the diversity of opisthobranchs known from the Indo-Pacific tropics. Records of previously described species were gleaned from a wide variety of literature sources. Data on shelled opisthobranch species were primarily obtained by means of Pilsbry's (1896) review of taxa and examination of *Zoological Record* subsequent to publication of Pilsbry's work. Nudibranch taxa were reviewed by Russell (1971, 1986) and through *Zoological Record.* Names of other opisthobranch taxa were obtained through *Zoological Record* and through perusal of primary literature and systematic reviews. It is widely known that *Zoological Record* has historically missed at least 20% of the molluscan names published (Bouchet and Rocroi, 1992). Every effort was made to survey the literature as thoroughly as possible, although it is likely that some taxa have been overlooked.

Taxa found were entered into a database using FileMaker Pro 2.1 (Claris Corp.). Every attempt has been made to incorporate as complete distributional data as are available. Species have been placed in synonymy according to the most recent published systematic treatment of that taxon and distributional data for junior synonyms have been combined with the known range of the senior synonym. Unpublished distributional records, based upon collections made in recent years from Aldabra Atoll, Madagascar, Malaysia, Papua New Guinea, the Philippines, and the Hawaiian Islands, were also incorporated into the database. Also included are unpublished records from Guam based on material collected by Clay Carlson and Patty Jo Hoff and unpublished records of material collected from Okinawa by Robert Bolland. Unpublished records from the Hawaiian Islands based upon collections of Pauline Fiene-Severns and Cory Pittman are included.

In addition to the described taxa represented in the above published literature, all known, but presently undescribed, taxa have been added to the database, with as complete distributional data as are known.

RESULTS

STANDING BIODIVERSITY OF OPISTHOBRANCHS

Boss (1971), in his review of the biodiversity of mollusks, estimated a global diversity of about 3,000 known species of opisthobranchs. From the published and unpublished records cited above, we have documented more than 3,400 species of opisthobranchs from the Indo-Pacific tropics. Of these, 1,019 are undescribed species. From these data, several conclusions can be drawn. The standing biodiversity of opisthobranchs worldwide is much higher than that estimated by Boss. Indo-Pacific opisthobranch diversity alone exceeds what was estimated for the whole world. A large proportion (more than 30%) of the species known to occur in the Indo-Pacific tropics are presently undescribed. Probably many other undescribed opisthobranch species have yet to be discovered within the Indo-Pacific and the estimates presented above represent the minimum percentage of undescribed species. This suggestion is supported by recent collection data from several localities throughout the Indo-Pacific. On a recent collecting trip to Tanzania, 11 undescribed opisthobranchs that had not been recorded from any other locality were documented in 11 days of observation. More recently, 20 completely novel, undescribed species were collected from the shallow waters of Luzon and Mindoro Islands in the Philippines within a 14-day period. Most of these were collected at specific localities that had been well-sampled by the same investigators employing the same sampling techniques over a period of several years. Many of these were large, brightly colored species that would not have been easily overlooked if they had been present during previous trips. This notion is further supported by the fact that some of the undescribed species found on previous trips were not located on subsequent trips, despite repeated intensive searching of the specific localities and habitats where they were originally encountered. Such data suggest that many of these species are sporadically encountered and probably have normally small populations.

Based on data presented in Table 1, approximately 40-50% of the species that occur in the better-known portions of the Indo-Pacific are undescribed. Using methods of estimating diversity for the entire Indo-Pacific by extrapolation as suggested by Colwell and Codington (1994) for terrestrial systems, it is likely that 4,000-4,800 species of opisthobranchs occur in the Indo-Pacific tropics. Perhaps

Table 1. Opisthobranchs from different Indo-Pacific localities.

Locality	# of species	% undescribed
Tanzania	258	16%
Madagascar	168	19%
Philippines	563	37%
Papua New Guinea	646	52%
Guam	474	47%
Hawaii	430	41%

twice that number occur in the world's oceans.

Ghiselin (1992) reported that the opisthobranch fauna of the Hawaiian Islands was one of the better-known ones in the Indo-Pacific and noted that 244 species were known. Recent intensive collections (largely through the efforts of Pauline Fiene-Severns and Cory Pittman) in the Hawaiian Islands raises the known fauna to 430 nominal species. This marked increase is reflective of the incomplete knowledge of species composition of opisthobranchs for any Indo-Pacific locality, including the more well-studied regions. In addition, it suggests that the Hawaiian opisthobranch fauna is not as impoverished or as attenuated as suggested by previous workers.

The percentage of the known fauna of specific localities in the Indo-Pacific which is undescribed is another measure of the completeness of knowledge of the biota of those particular localities. The total number of known species and the percentage of undescribed taxa from six Indo-Pacific localities is provided in Table 1. While these figures suggest that western Indian Ocean localities (Tanzania and Madagascar) have fewer species and a smaller percentage of undescribed species, it is likely that both are artifacts of less intensive collecting.

Another way in which the state of knowledge of the systematics of Indo-Pacific opisthobranchs can be assessed is by comparing proportions of undescribed versus described species of particular taxa. In several groups of opisthobranchs (Table 2), the proportion of undescribed species ranges from 22-58%. This closely resembles the range of geographic- rather than taxon-based comparisons, presented above.

Whatever the measure of biodiversity of Indo-Pacific opisthobranchs, the conclusion that many undescribed species are present throughout the region remains. Yet undiscovered taxa are likely to continue to be found by future exploration, thus increasing the estimated biodiversity of the region.

KNOWN DISTRIBUTIONS OF INDO-PACIFIC OPISTHOBRANCHS

Estimating the known distributions of Indo-Pacific opisthobranchs is an extremely difficult undertaking, as lack of data is usually reflected by apparently narrow distributions. For species that are large, conspicuous in their coloration, and fairly abundant, it is far more likely that presently known distributional data reflect biogeographical reality. In other cases, where information is far more scanty, widely disjunct distributions are known. For example, the aeolid nudibranch, *Flabellina macassarana* Bergh, 1905, was originally described from a single specimen from the Macassar Strait in Indonesia. There are no other published records of this species. Recently, single specimens were collected in the Philippines and Tanzania. All three known specimens have been found in about 30 m depth and the two recently discovered ones have been found in association with a single species of the hydroid genus, *Eudendrium*. *F. macassarana* is a highly specialized predator which occurs at considerable depth and appears to be uncommon even in its preferred habitat. All of these factors contribute to the lack of distributional data. Nevertheless, it is widespread, ranging from the western Indian Ocean to at least the western Pacific. Additional records may fill in the gaps and possibly extend the known range farther eastward. Known distributions at least permit making inferences regarding the minimum known range for a species.

Distributional data from specific localities can also provide evidence to infer the relative state of knowledge of that biota and of adjacent ones. On a recent collecting trip to the Philippines, 218 opisthobranch species were observed in two weeks of sampling. Of these, 61 had not been previously recorded from the Philippines. This is despite the fact that most of the areas where collecting took place had been repeatedly sampled over a several-year period. Similarly, of the 127 species collected from Tanzania in 11 days, more than half (67) had not been previously recorded from the coast of eastern Africa, including 35 species which had not been found previously in the Indian Ocean.

All of these measures of distributional pattern indicate that the distribution of opisthobranch species in the Indo-Pacific remains poorly known. Despite gaps in distributional data, present distributions can serve as estimates of geographical limits for species known from widely separated localities. The exploration of areas that are poorly known as well as continued observation of areas which are better known is fundamental to building a basic understanding of Indo-Pacific opisthobranch distributions.

Table 2. Percentage of undescribed species within five genera of Indo-Pacific opisthobranchs.

Taxon	# of species	# of species undescribed	% of species undescribed
Chelidonura	24	8	33%
Chromodoris	164	36	22%
Hypselodoris	39	19	48%
Nembrotha	29	7	24%
Cuthona	114	66	58%

LEVELS OF ENDEMISM IN INDO-PACIFIC OPISTHOBRANCHS

The percentage of species which are endemic to a particular region is a measure of its geographical isolation and is often reflective of isolating mechanisms that have permitted allopatric speciation, in the absence of gene flow. By definition, species are considered endemic to a region if they are known from nowhere else. Consideration of endemism is independent of the size of the region. Thus, a species can be considered endemic to a particular archipelago, while another more widespread species can be considered endemic to the Indo-Pacific. However, in the absence of sufficient distributional data, erroneous assumptions of endemism can be made. In the above example, *Flabellina macassarana* might have been considered endemic to Indonesia, prior to its discovery from other widespread localities. Given the present level of understanding of Indo-Pacific opisthobranch distributions, assumptions of endemism should be made with extreme caution. Species known only from their original description are especially suspect as being endemic.

Taxonomic considerations may also impact concepts of endemism. Within the Indo-Pacific tropics, the Hawaiian Islands represent one of the most isolated archipelagos. They are isolated from both continental sources and other island groups. In the early to mid-twentieth century, most species found in the Hawaiian Islands were considered to represent new species and were generally considered endemic to the archipelago. With taxonomic refinement and collection of additional taxa, the perceived percentage of endemic opisthobranchs has diminished over time (Table 3). However, taxonomic refinement can instead increase estimated levels of endemism as cryptic and sibling species are recognized.

Present estimates of levels of endemism of Hawaiian opisthobranchs vary widely (from 4-43%) depending on the manner in which endemism is calculated. If all species known only from the Hawaiian Islands are considered "endemic," then 43% of the taxa are apparently restricted to the Hawaiian Islands. Alternatively, one could take a far more conservative approach. Species that are fairly large, brightly colored, and commonly encountered are less likely to overlooked from other localities. When only conspicuous and common Hawaiian species are considered, the resulting level of endemism (4%) is an order of magnitude lower than literal assumptions of endemism. The more conservative estimate appears far more likely to represent biogeographical reality.

Endemism elsewhere in the Indo-Pacific tropics remains even more poorly known than it is in the Hawaiian Islands. Endemism in subtropical areas adjacent to strictly tropical regions such as southeastern and western Australia (Rudman, 1987; Wells and Bryce, 1993), Japan (Baba, 1949, 1955) and South Africa (Gosliner, 1987a) appears to be high. This perception is difficult to quantify, given the incomplete state of biogeographical knowledge of these regions.

Table 3. Percent endemism of Hawaiian opisthobranchs.

Publication	% endemism
Pease, 1860	90%
Pilsbry, 1921	88%
Ostergaard, 1955	89%
Kay, 1979	29%
Gosliner, unpublished	13%
Gosliner, present study	4.5% - 43%

It appears that some strictly tropical regions of the Indo-Pacific also exhibit some degree of endemism, as well. Present data suggest that the western Indian Ocean and the Red Sea contain species that are not found elsewhere in the Indo-Pacific. For example, Rudman (1987) discussed several species pairs of chromodorid nudibranchs, in which one sister species is restricted to the western Indian Ocean and its hypothesized sister species is found in the Pacific. These species generally have similar but divergent color patterns. The circumstantial evidence that these taxa are sister species is compelling, despite the fact that explicit phylogenies are not known for any of these taxa.

Apparent regional endemism within the Indo-Pacific is not restricted to marginal portions of this vast area. It appears that many species in the western Pacific are restricted to this area, in the region of highest diversity extending from the Philippines southward to Indonesia and Papua New Guinea. However, the systematics and distribution of taxa within this subregion remain poorly known. Consequently, levels of endemism of geographical restriction within the subregion remain largely unstudied.

While regional endemism within portions of the Indo-Pacific appears likely, there are even fewer examples of more restricted endemism to a single island or portions of archipelagos. Some Hawaiian endemics appear to be more restricted. Two undescribed chromodorids which are large and brightly colored are commonly encountered in the waters of leeward atolls such as Kure and Midway, but are apparently absent from the high islands of the chain. Other species are apparently restricted to certain islands of the western Indian Ocean, but absent from others. For example, *Siphopteron michaeli* (Gosliner and Williams, 1988), is known from Reunion Island, but is apparently absent from Mauritius, Madagascar, and the African mainland.

Major gaps exist in the systematics and distribution of opisthobranch species inhabiting the Indo-Pacific tropics. Nevertheless, several conclusions can be drawn. Levels of endemism differ widely between terrestrial and marine environments of the same islands. Suggestions that these are taxonomic artifacts are not supported by increased disparity between Hawaiian land and marine environments with increased taxonomic refinement.

Other patterns of regional endemism appear to occur within Indo-Pacific opisthobranchs. Ascertaining levels of endemism for these regions or circumscribing the areas of endemism remain difficult owing to the absence of primary distributional and systematic data for most taxa and regions.

VICARIANCE BIOGEOGRAPHY IN INDO-PACIFIC OPISTHOBRANCHS

Vicariance biogeography focuses upon the study of phylogenetically related taxa, preferably sister taxa, which exhibit geographical separation from each other. The intent of these studies is to identify patterns of vicariance (or geographical separation) within and between clades, in order to recognize isolating mechanisms restricting gene flow and permitting speciation. As in the case of classical biogeography, taxonomy and distributional ranges of component taxa must be known. Additionally, vicariance biogeographical studies require hypotheses of phylogeny for the taxa being considered. In the case of Indo-Pacific opisthobranchs, this usually requires species-level phylogenies, because few higher taxa exhibit endemism within the regions (Gosliner, 1992). Few species-level phylogenies have been hypothesized for clades of opisthobranchs that inhabit the Indo-Pacific tropics. Phylogenetic studies of opisthobranchs which inhabit the region are restricted to those of the Gastropteridae (Gosliner, 1989), the Flabellinidae (Gosliner and Willan, 1991), *Hallaxa* (Gosliner and Johnson, 1994), and *Thuridilla* (Gosliner, 1995).

Another requisite element of vicariance biogeography is that sister taxa be geographically isolated from each other. This may mean classical geographical isolation such as construction of physical barriers (such as the Isthmus of Panama completely separating the Caribbean Sea from the eastern Pacific Ocean) or other barriers such as changes in oceanic current patterns or other factors which may significantly reduce gene flow between localities, thus permitting differentiation of isolated populations. When the above phylogenetic hypotheses are examined for geographical isolation of sister taxa, it is evident that there are few cases where sister taxa are allopatric. In the vast majority of cases, sister taxa have at least some area of geographical overlap and in many instances their entire ranges are almost completely sympatric (Gosliner and Willan, 1991; Gosliner, 1995). As vicariance biogeography assumes that speciation must be preceded by geographical separation, such sympatric distributions can be explained only by dispersal subsequent to speciation.

The relative failure of the above studies to demonstrate vicariance suggests that many taxa where subsequent dispersal has occurred are not informative for vicariance biogeographical studies. This is a common problem in the study of Indo-Pacific marine organisms, one which has led many investigators to suggest that it may be impossible to determine patterns of vicariance in Indo-Pacific taxa (see Kabat, this volume). However, it is apparent that some opisthobranch taxa do indeed exhibit endemism within the Indo-Pacific. Members of the genera *Halgerda, Chromodoris, Hypselodoris,* and *Nembrotha* appear especially fruitful for these studies, as they appear to contain species with distributions that are more restricted than many other taxa.

DISCUSSION AND CONCLUSIONS

The above data suggest several modifications of the conventional view concerning tropical opisthobranch diversity. Biodiversity of Indo-Pacific opisthobranchs is much higher than previously thought. Some of this is due to incomplete compilation of described taxa, but the bulk of increased diversity stems from the fact that at least 30% of the species are presently undescribed. Based on recent collections, many additional undescribed taxa likely remain to be discovered.

Primary distributional data are woefully lacking for both Indo-Pacific taxa and faunistic data for specific localities. The known opisthobranch fauna of the Hawaiian Islands has increased by more than 75% in the last three years. This, combined with the fact that the Hawaiian Islands are one of the better known portions of the Indo-Pacific, is indicative of the inadequate state of the knowledge of the entire region. Certainly, much additional data must be collected before a coherent picture of Indo-Pacific opisthobranch distributions can be presented.

Nelson and Platnick (1981) have suggested that the perceived level of marine endemism of the Hawaiian Islands would rise to approach the highly endemic terrestrial biota (± 90%) with greater taxonomic refinement. Gosliner (1987b) disputed this claim, stating that the consequences of larval dispersal of marine organisms differs fundamentally from the more limited dispersal of the terrestrial biota. Regardless of the variation in the currently perceived levels of endemism of the Hawaiian opisthobranch fauna, it is clear that the estimates are moving in the opposite direction with taxonomic and biogeographical refinement. These data strongly suggest that levels of endemism for marine taxa do differ from freshwater and terrestrial biotas and that differences in dispersal capabilities are responsible for these observed differences. Dispersal can affect endemism both prior to and following speciation. Initially, dispersal is essential in maintaining gene flow between separated populations of species, thus reducing the likelihood of speciation. Secondarily, if speciation has occurred as a result of geographical isolation, then subsequent dispersal may mask the original vicariance that permitted speciation.

Levels of endemism clearly differ between the terrestrial and freshwater and the marine environments. These differences do not appear to be taxonomic artifacts, but rather reflect different isolating mechanisms, different geographical barriers, and different dispersal capabilities. In other words, marine biotas appear to have evolutionary histories that are clearly separate from corresponding biotas associated with land masses. One exception to this general rule is the coastal strand vegetation of Indo-Pacific terrestrial environments. Plants inhabiting these regions have seeds that can float in sea water for extended periods and maintain their viability (Carlquist, 1974). The seeds of these plants are thus distributed by the same ocean surface currents that disperse marine larvae. Consequently, levels of endemism of strand vegetation are far lower than upland floras of the same islands. One difference between plant seeds and marine larvae is that most marine larvae are capable of undergoing vertical migration in and out of current and eddy systems, while seeds remain at the surface throughout their time in sea water. Nevertheless, the net effect of lower endemism in more readily dispersed taxa remains the same and is in sharp contrast to terrestrial biotas that are absent from the immediate shoreline.

There are few phylogenies of opisthobranch taxa with Indo-Pacific representatives. The available studies demonstrate little vicariance of sister taxa. Springer (1982) suggested the margin of the western Pacific Plate provided a historical geographical barrier for Indo-Pacific marine taxa and that sister species of taxa found on the Australasian Plate are likely restricted to the non-marginal portions of the Pacific Plate. None of the opisthobranchs for which there are hypothesized phylogenies exhibit this pattern, including the relatively few species that appear to have distributions that are vicariant with sister taxa.

Relatively few opisthobranchs have distributions which are widespread on the non-marginal portions of the Pacific Plate. One of the only examples of this pattern of distribution is an undescribed species of *Chelidonura*, which is thus far known from only the Hawaiian Islands and Easter Island. However, no hypothesis of phylogeny has been suggested the most likely sister species of this taxon. It is likely that other Indo-Pacific species could be found from more than one locality on the Pacific Plate, but it is premature to make that assessment for most taxa, owing to incomplete distributional data. It is also plausible to assume that some of the species endemic to particular islands of the Pacific Plate have sister taxa that are restricted to the Indian Ocean Plate. However, sufficient distributional and phylogenetic data are presently lacking to generalize whether this is a common pattern for Indo-Pacific opisthobranchs.

The systematics, biogeography, and phylogeny of Indo-Pacific opisthobranchs are all poorly understood. Recent evidence of previously undetected taxa and of distributions which are markedly more widespread than previously indicated, are strongly suggestive that many fundamental data are lacking. Additional intensive collections from many localities are essential to provide the necessary data for comprehensive biogeographical studies of Indo-Pacific opisthobranchs.

Although there is a paucity of biogeographical data for Indo-Pacific opisthobranchs, species level phylogenies of opisthobranchs, fundamental for vicariance biogeographical studies, are even less well-known. Despite the necessity for additional species descriptions and more detailed distributional data, the lack of phylogenetic hypotheses is the weakest link in building an understanding of vicariant patterns for Indo-Pacific opisthobranchs. While documenting the diversity and its distribution within the Indo-Pacific is certainly necessary, increasing knowledge of phylogeny is of paramount importance to understanding vicariant distributional patterns within the region.

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LITERATURE CITED

Baba, K. 1949. *Opisthobranchia of Sagami Bay Collected by His Majesty, the Emperor of Japan.* Tokyo, Iwanami shoten. 185 pp.

Baba, K. 1955. *Opisthobranchia of Sagami Bay collected by His Majesty, the Emperor of Japan. Supplement.* Tokyo, Iwanami shoten. 59 pp.

Boss, K. J. 1971. Critical estimate of the number of recent Mollusca. *Occasional Papers on Mollusks* 3:81-135.

Bouchet, P. and J.-P. Rocroi. 1992. Supraspecific names of molluscs: a quantative review. *Malacologia* 34:75-86.

Carlquist, S. 1974. *Island Biology.* New York, Columbia University Press. 660 pp.

Colwell, R. K. and J. A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society of London. B. Biological Sciences* 345:101-118.

Ghiselin, M. T. 1992. How well known is the opisthobranch fauna of Madang, Papua New Guinea? *Proceedings of the Seventh International Coral Reef Symposium* 2:697-701.

Gosliner, T. M. 1987a. *Nudibranchs of Southern Africa. A Guide to the Opisthobranch Molluscs of South Africa.* Monterey, Sea Challengers. 136 pp.

Gosliner, T. M. 1987b. Biogeography of the opisthobranch gastropod fauna of southern Africa. *American Malacological Bulletin* 5(2):243-258.

Gosliner, T. M. 1989. Revision of the Gastropteridae (Opisthobranchia: Cephalaspidea) with descriptions of a new genus and six new species. *The Veliger* 32(4):333-381.

Gosliner, T. M. 1992. Biodiversity of tropical opisthobranch gastropod faunas. *Proceedings of the Seventh International Coral Reef Symposium* 2:702-709.

Gosliner, T. M. 1995. The genus *Thuridilla* (Opisthobranchia: Elysiidae) from the tropical Indo-Pacific, with a revision of the phylogeny and systematics of the Elysiidae. *Proceedings of the California Academy of Sciences* 49(1):1-54.

Gosliner, T. M. and S. Johnson. 1994. Review of the genus *Hallaxa* (Nudibranchia: Actinocyclidae) with descriptions of nine new species. *The Veliger* 37(2):155-191.

Gosliner, T. M. and R. C. Willan. 1991. Review of the Flabellinidae (Nudibranchia: Aeolidacea) from the tropical Indo-Pacific, with the descriptions of five new species. *The Veliger* 43(2):97-133.

Kay, E. A. 1979. *Hawaiian Marine Shells.* Honolulu, Bishop Museum Press. 653 pp.

Nelson, G. and N. Platnick. 1981. *Systematics and Biogeography. Cladistics and Vicariance.* New York, Columbia University Press. 567 pp.

Ostergaard, J. M. 1955. Some opisthobranchiate Mollusca from Hawaii. *Pacific Science* 9(2):110-136.

Pease, W. H. 1860. Descriptions of new species of Mollusca from the Sandwich Islands. *Proceedings of the Zoological Society of London* 1860 (2):18-37.

Pilsbry, H. A. 1896. Philinidae, Gastropteridae, Aglajidae, Aplysiidae, Oxynoidae, Runcinidae, Umbraculidae, Pleurobranchidae. *Manual of Conchology* 16(61-64, 64a):1-262, pls. 1-74.

Pilsbry, H. A. 1921 (1920). Marine mollusks of Hawaii, xiv-xv. *Proceedings of the Academy of Natural Sciences of Philadelphia* 72:360-382.

Rudman, W. B. 1987. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris epicuria, C. aureopurpurea, C. annulata, C. coi,* and *Risbecia tryoni* colour groups. *Zoological Journal of the Linnean Society* 90:305-407.

Russell, H. D. 1971. *Index Nudibranchia.* Greenville, Delaware Museum of Natural History. 141 pp.

Russell, H. D. 1986. Index Nudibranchia. Supplement I. *Special Occasional Publication, Harvard University Mollusk Department* 7:1-100.

Springer, V. 1982. Pacific plate biogeography, with special reference to shorefishes. *Smithsonian Contributions to Zoology* 367:1-182.

Wells, F. E. and C. W. Bryce. 1993. *Sea Slugs of Western Australia.* Perth, Western Australian Museum. 184 pp.

Winston, J. 1988. The systematists' perspective. *In: Biomedical Importance of Marine Organisms,* D. Fautin, ed. *Memoirs of the California Academy of Sciences* 13:1-6.

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Dynamic clams: changes in the bivalve fauna of Pacific islands as a result of sea-level fluctuations

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Abstract: Sea-level fluctuations continually alter the distribution and nature of shallow-water environments, although not all habitats are equally affected. Shallow-water habitats on coral reefs around oceanic islands can be divided into markedly different inner- and outer-reef systems. During regressions, the former are stranded while the latter persist. Previously I showed that numerous species are restricted to inner-reef habitats; I predicted that these would undergo local extinction across most of the central Pacific Ocean during regressions, and would expand back into the region during high sea stands. An examination of the fossil record of bivalves on Niue and other central Pacific islands provides support for both of these hypotheses, and shows that the range of some inner-reef specialists can vary substantially among high sea stands. Despite such unstable ranges, limited data do not indicate higher global extinction rates for inner-reef specialists. Sea-level fluctuations can provide vicariant opportunities for speciation, but also impede the potential for geographic differentiation of populations of inner-reef specialist taxa, because the lifespan of insular populations is often limited to the duration of single high sea stands.

To understand the origins of the present distribution and diversity of organisms, one must consider the dynamics of species ranges. Are species ranges relatively stable through time or fluctuating? What factors influence the relative stability of species distributions, and what are the evolutionary consequences of varying degrees of range stability? Climatic cycles caused by orbital variation (Milankovitch cycles) are perhaps the most important agents affecting species distributions, and operate on the time scale between ecological and evolutionary time (Bennett, 1990). Such cycles have resulted in major changes in species distributions and consequently in community composition on land (*e.g.* Davis, 1981; Webb, 1987). In marine habitats climatic cycles are manifested in a variety of ways, including changes in temperature, patterns of ocean circulation, and large-scale fluctuations in sea level. Here I examine how changes in sea level have affected the stability of insular bivalve populations, and consequently the distribution of bivalve species in the central Pacific Ocean. I also consider how range stability can influence extinction and speciation in the area.

Sea-level fluctuations lead to striking changes in shallow-water environments, affecting the extent, nature, and distribution of a variety of habitats. The reef geomorphology of central Pacific islands is such that the effects of sea-level fluctuations are particularly clear. Atolls, as well as fringing and barrier reefs around volcanic islands, can each be categorized into outer- (= fore) and inner-reef components; these components are markedly different in physiography, habitats available, and fate during sea-level fluctuations (Paulay, 1990).

Inner-reef systems, including mangals, reef flats, moats, and lagoons, are separated from the outer reef slope by a shallow, usually intertidal reef margin that often rises at its seawardmost point to form a reef crest. Among islands, inner reefs vary greatly in extent, from narrow, intertidal reef pavements to atoll lagoons hundreds of km^2 in area. The degree to which they connect with the ocean also varies: from lagoons that are brackish to hypersaline ponds, having no surface connections to the ocean, to those that communicate via wide, deep passages and consequently have oceanic qualities (Salvat, 1967; Chevalier, 1979). Most inner-reef systems are dominated by soft sediments, with grain size ranging from mud to sand to reef rubble, depending primarily on the size of the reef system and its connectivity with the open ocean. Because even the deepest lagoons are shallower than the greatest regression during a glacial cycle (Stoddart, 1973), inner-reef habitats are periodically stranded in their entirety, and exhibit a variety of intermediate stages at other stages of the sea-level cycle (Paulay, 1991). In contrast, outer reef slopes are steep, narrow fringes seaward of inner reefs, show much less variation among islands, and are dominated by hard substrata. Sea-level fluctuations only displace outer reefs up or down slope.

Tectonic processes are important in changing the configuration of reef systems, and are ultimately responsible for the origin of islands and their development from

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young volcanoes to atolls and seamounts (Darwin, 1842; Grigg, 1982), however, they operate over much longer timescales than do sea-level fluctuations on mid-plate islands (Paulay and McEdward, 1990). Further, although the impact of tectonic processes on individual islands is marked, their effects are not synchronized among islands. Thus unlike eustatic sea-level fluctuations, tectonic changes do not cause basin-wide changes in habitat.

The marked differences in habitats between outer and inner reefs are reflected in their biota: each environment supports many species that are restricted to it. Among bivalves, about one-third of the central Pacific species appear to be restricted to inner-reef habitats. I would expect such species (= "inner-reef specialists") to undergo local extinction when regressions strand their habitats, while species that occur on outer reefs ("outer-reef inhabitants") persist (Paulay, 1990). If such is the case, inner-reef specialists could have highly dynamic ranges that expand and contract as sea levels rise and fall. Their populations could survive in refugia during low sea stands, re-expanding to the Micronesian and Polynesian islands of the Pacific Plate during each high stand. Likely refugia include islands of the Melanesian arc (New Guinea - Solomons - Vanuatu - Fiji; Fig. 1) and continental islands to the west: these areas have complex reef systems, where inner reef-like habitats persist at all sea stands (Paulay, 1990).

Here I use the fossil record of bivalves on Niue and other central Pacific islands to test the hypotheses (1) that regressions cause the extinction of inner-reef specialists but not of outer-reef inhabitants, and (2) that inner-reef specialists experience the predicted range contractions and expansions in the central Pacific through successive sea-level cycles. I also look at the long-term consequences of stability versus turnover for insular populations. After reviewing the relevant insular setting and methods, I discuss each of these three points in turn.

INSULAR SETTING

The effects of sea-level fluctuations can be examined on islands that have been uplifted by tectonic forces, and thus have experienced a recent, localized (isostatic) sea-level fall. One can compare the fossil fauna of inner reefs that formed prior to uplift, when the island experienced a localized high-sea stand, with the living fauna surviving on the emergent island at present. Such uplifted limestone islands are uncommon on the largely subsiding Pacific Plate, and include (1) islands raised by lithospheric flexure around new volcanic loads in the Hawaiian, Tuamotu, Pitcairn, Austral, and Cook Island groups; (2) islands apparently raised by lithospheric arching prior to subduction (Niue, Fais); (3) a few islands whose emergence is poorly understood (Banaba, Nauru) (Dubois *et al.*, 1975; McNutt and Menard, 1978; Pirazzoli and Montaggioni, 1985; Spencer *et al.*, 1987). Tectonic uplift is common

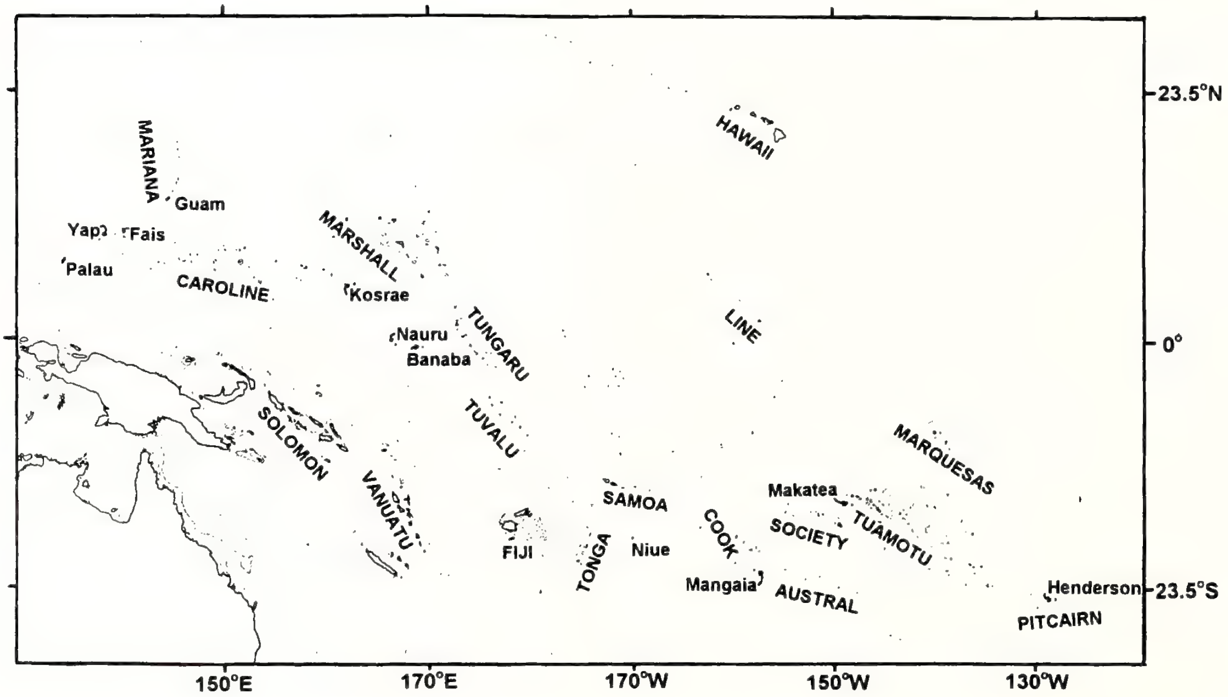

Fig. 1. Map of Pacific islands. Island names in lower case, island group names capitalized.

along the island arcs that line the western plate margin (Mariana - Palau - Melanesian Arc - Tonga), and some of these arc islands (Solomons, Vanuatu) were secondarily accreted onto the Pacific Plate.

Six limestone islands that formed on the Pacific Plate lack exposed volcanics and could be uplifted atolls: Nauru, Banaba, Fais, Makatea, Niue, and Henderson. The first three are poorly known, and together with Makatea have been mined for phosphate - an activity which disturbs paleolagoonal deposits considerably. Makatea has received more attention than the first three. However the atoll, apparently Miocene in age, has suffered from considerable diagenesis as well as mining (Montaggioni, 1985). Both Niue and Henderson sustain well-preserved lagoonal deposits with rich fossil communities dating from the Pliocene and Pleistocene respectively (Paulay and Spencer, 1988, 1992; Spencer and Paulay, 1989).

Niue lies 400 km east of the Tongan island arc (Fig. 1). The island's uplift is thought to be the result of arching of the Pacific Plate prior to its subduction into the Tongan Trench (Dubois *et al.*, 1975). The core of Niue comprises an uplifted atoll with the original atoll form well preserved: a ring of reef limestones reaching an elevation of 68 m encircles a lagoonal depression (34 m) of calcarenites (Schofield, 1959; Paulay and Spencer, 1992). Surface deposits in the lagoon are of late Pliocene age [(1.7)-2.0-2.2 Ma; dates based on Sr isotope ratios, P. Aharon, pers. comm., 1995]. While the peripheral core atoll reef facies has undergone considerable diagenesis and has generally poor fossil preservation, the molluscan fauna of the central lagoon is well-preserved at many locations. Several marginal, Pleistocene apron reefs have been accreted onto the atoll core since initiation of uplift, some forming parts of the terraces that surround the island. These younger reefs have additional, often very well-preserved, molluscan assemblages.

Since uplift, a limited inner reef has developed around Niue: a narrow reef platform especially pronounced on the leeward half of the coastline. The reef platform is an intertidal to shallow subtidal (< 0.5 m, except for a few deeper pools) reef pavement with pockets of coarse sediments, fronted by a well-developed intertidal reef crest. Eight species of inner-reef-specialist bivalves [*e. g. Fragum mundum* (Reeve, 1845), *Isognomon perna* (Linné, 1767), *Isognomon* cf. *laticostata* (Reeve, 1858)] occupy this habitat (Paulay, 1990 and unpub.). Much of the windward coast has no inner-reef development, or has only narrow (< 10 m), elevated (> 1 m above high-water level) splash pools.

The outer reef slope around Niue generally begins as a steep incline, meeting a gently sloping, sandy terrace (ca. 50-100 m wide) at 25-50 m depth. In some embayments (at Alofi and Avatele), limited submarine terracing occurs also at shallower depths. Despite these terraces, the 100 m isobath lies within 200-400 m of the reef crest (Schofield, 1959). As on most central Pacific islands, the outer reef slope is dominated by hard substrata, with mobile sediments limited to sand pockets (often substantial) in reef grooves, and to the sand- and rubble-dominated terrace at 25-50 m depth.

MATERIALS AND METHODS

In 1986 and 1991, I collected fossils at 19 sites in the fossil atoll lagoon and 21 sites in the surrounding core and apron reef facies, selecting sites for their accessibility and fossil preservation. Most of the lagoonal sites were at calcarenite quarries: bulldozed pits used in supplying sand for construction. Active and abandoned quarries of unconsolidated calcarenites are scattered throughout Niue's interior, many of them noted on the Map of Niue (NZMS, 1985). The vertical extent of quarries varies from ca. 1 to 8 m, with most in the 1-3 m range; their horizontal extent ranges from a few tens to hundreds of meters. A few quarries show striking vertical changes in fossil assemblages and diagenesis; this, together with aspects of intersite faunal variation (Paulay, unpub.) and a spread in Sr isotope dates (Wheeler and Aharon, 1991; Aharon *et al.*, 1993; P. Aharon, pers. comm., 1995), indicates that lagoonal faunas exposed in quarries likely date from more than one high sea stand. Most of the sediments now found on the lagoon surface were apparently deposited over only a few high sea stands during the late Pliocene, judging from general faunal similarity, limited vertical spread (< 25 m across the entire lagoon), and relatively narrow range of Sr dates [(1.7)-2.0-2.2 Ma; P. Aharon, pers. comm., 1995].

I sampled the recent fauna by SCUBA on the outer reef slope and by snorkeling and reef walking on the reef flat. Results of the 1986 survey are listed in Paulay (1990); the 1991 survey added several new records. The single bivalve species [*Fragum fragum* (Linné, 1758)] recorded from Niue by Cernohorsky (1970; and relisted in Paulay, 1990) is here removed from the faunal list: in my extensive surveys, I did not find *F. fragum*, appropriate habitat for which is absent on the island. The similar *F. mundum* inhabits reef flats on Niue. As previously noted (Paulay, 1987), several of Cernohorsky's bivalve identifications (including *Fragum* identifications) in a similar list published on the fauna of the neighboring Cook Islands have proved to be incorrect.

I used the extensive Indo-West Pacific collections and library resources of the U. S. National Museum of Natural History (USNM) to aid in taxonomic work. T. R. Waller (USNM) kindly identified the pectinid species. I identified fossil specimens that matched living species as such, and labeled those that were close to living forms but differed slightly but consistently from the range of variation exhibited by Recent specimens with "cf." I noted those fossils that differed markedly enough from living species to be considered specifically distinct with "aff." Several of the latter could be ancestral to Recent species. Specimens that were not sufficiently well-preserved for a definitive identification but otherwise matched a Recent species were labeled with "?". Fossils belonging to fairly well-known groups that did not match or approximate any living forms encountered in collections or the literature, I assumed to be globally extinct. The global survival (extinct or extant) of species that belong to poorly known taxa (mostly micro-lucinids) was left unresolved. I listed species too poorly preserved for specific identification under the lowest category to which they were identified and without numerical species designation. Although I listed the taxonomically poorly known and poorly preserved species (Table 1), these were not included in the analyses below. A taxonomic review of the fossil fauna will be presented elsewhere.

RESULTS AND DISCUSSION

TURNOVER AND STABILITY ON NIUE

I recognize 104 species of fossil bivalves on Niue: 83 from the fossil lagoon, nine from probable atoll-core fore-reef facies encircling the lagoon, and 30 from marginal mid-late Pleistocene reefs (Table 1). The stratigraphic demarcation between Pliocene atoll-core fore-reef facies and marginal, older Pleistocene apron reefs (which could have been secondarily accreted onto the core after initiation of uplift) is poorly understood. Unless otherwise noted, all discussion below concerns the Pliocene lagoonal fauna. Twelve of the 83 lagoonal species are either very poorly preserved, or belong to taxonomically poorly known groups, and will not be further considered unless otherwise noted (Table 1).

Among the remaining 71 lagoonal species, 20 (28%) appear to be globally extinct. Six of the 20 (*Excellichlamys* aff. *spectabilis*, *Laevichlamys* aff. *squamosa*, *Tellina* aff. *crucigera*, *Wallucina* aff. *haddoni*, *Pillucina* aff. *spaldingi*, and *Corculum* aff. *cardissa* are close, and might be ancestral, to the Recent species named; thus their disappearance could represent pseudoextinction. Considering the possibility of pseudoextinction, the rate of global extinction for the fauna is 20-28%. This is comparable to the level of extinction experienced by Japanese and Californian bivalves of the same age, and is similar to background levels of extinction for the class (Stanley *et al.*, 1980). There is thus no evidence for any major extinction events affecting the fauna since the late Pliocene. In addition to the six species noted above, two additional ones (*Fragum* cf. *fragum, Timoclea* cf. *marica)* show limited morphological disparity from Recent populations. Thus despite their characteristically slow rate of evolution (Simpson, 1953; Stanley, 1979), up to 14% (N = 57) of the surviving bivalve species in the fauna could have undergone morphological change since the latest Pliocene.

Local extinction among the 51 extant lagoonal species was 62%. Is this high extinction rate due to changes in habitat or to a general instability of species distributions on isolated oceanic islands? Data on taxonomic affinity, microhabitat use, and zonation indicate that regression-caused habitat change was the predominant cause of local extinction.

The Pliocene and Recent habitats compared are quite different: the Pliocene's shallow lagoon, dominated by reef sands versus today's narrow reef flats and steep outer reef slope, dominated by hard substrata. This difference is reflected in the taxonomic composition of the fauna (Fig. 2, Table 2): taxa that prefer outer reef habitats (*e. g.* pectinoids) are proportionately better represented in the Recent fauna, while groups that prefer lagoonal soft bottoms (*e. g.* tellinoids) (Paulay, 1990) are better represented in the Pliocene fauna. Both the Pliocene and Recent faunas are composed solely of pteriomorph and heterodont (defined as in Boss, 1982, but not Waller, 1990, to include the few myoids) bivalves, as is typical of the shallow-water fauna of all central Pacific islands. The ratio of pteriomorphs to heterodonts is lower in the Pliocene lagoonal fauna than among species living on Niue today (0.57 versus 0.93), albeit not significantly so (G-test, $G = 2.52$, $p > 0.05$). The lower proportion of pteriomorphs in the Pliocene runs counter to a taphonomic bias favoring the preservation of the frequently calcitic shells of pteriomorphs over the almost strictly aragonitic shells of heterodonts. The difference between Pliocene and Recent pteriomorph / heterodont ratios could reflect differences in microhabitat use: while the majority of pteriomorphs prefer hard substrata, the majority of heterodonts live in soft sediments. Thus in South Polynesia, 92% of pteriomorphs and 23% of heterodonts live on or in hard substrata (data from Paulay, 1990: appendix). Indeed, soft-bottom species are better represented (G-test, $G = 5.75$, $p < 0.05$) in the Pliocene than in the Recent fauna of Niue (Fig. 3). An analogous trend is found in the Recent fauna of South Polynesia: a significantly greater proportion of inner-reef specialists occur among soft-bottom than among hard-bottom species (Paulay, 1990).

On Niue, the contrasting fates of inner-reef specialists and outer-reef inhabitants support the hypothesis that the major cause of local extinction for the Pliocene fauna was uplift-associated habitat loss. The habitat specificities of all but four of the 51 extant species are known to some degree (Table 1). Inner-reef specialists suffered complete (100%, N = 20) local extinction with uplift; the limited inner-reef specialist assemblage inhabiting the reef flats of

Table 1. Fossil bivalves from Niue. ID (quality of identifications): +/+, species sufficiently well preserved to be identifiable and identified; +/-, species sufficiently well preserved to be identifiable but taxonomic problems with identification at present; -/-, species not sufficiently well preserved to be identifiable to species. Core Lagoon: found (1) or not found (0) in lagoonal facies in island interior. Core? Fore reef: found (1) or not found (0) in fore-reef facies judged to represent original core reef of island. Marginal Fore reef: found (1) or not found (0) in marginal, Pleistocene apron reefs secondarily accreted onto island since initiation of uplift. Globally extant?: globally extant (1), globally extinct (0). Locally extant?: locally extant (1), locally exinct (0). Range to W: known (1) or not known (0) from islands to west of Niue. Range to E: known (1) or not known (0) from southeastern Polynesia. Habitat: I, inner reefs only; O, outer reefs only; IO, inner and outer reefs.

Family	Species	ID	Core Lagoon	Core? Fore reef	Marginal Fore reef	Globally Extant?	Locally Extant?	Range to W	Range to E	Habitat
Arcidae	*Arca avellana* Lamarck, 1819	+/+	1	0	1	1	1	1	1	IO
	A. ventricosa Lamarck, 1819	+/+	1	0	0	1	0	1	1	I
	A. plicata (Dillwyn, 1817)	+/+	0	0	1	1	1	1	1	O
	A. sp. 1	+/+	1	0	0	0?	0	?	?	?
	Barbatia amygdalumtostum (Röding, 1798)	+/+	0	1?	1	1	0	1	0	IO
	B. foliata (Forsskål, 1775)	+/+	1	0	0	1	0	1	1	I
	B. ?parva (Sowerby, 1844)	+/+	1	0	0	1	1	1	1	IO
	B. ?setigera (Reeve, 1944)	+/+	1	0	0	1	0	1	1	IO
	B. sp. 1	+/+	0	0	1	1	1	1	0	IO
	B. sp. 8	+/+	1	0	0	0	0	?	?	?
	Hawaiarca rectangula (Dall, Bartsch and Rehder, 1938)	+/+	0	0	1	1	1	0	0	IO
	Anadara ?uropigimelana (Bory de St. Vincent, 1824)	+/+	1	0	0	1	0	1	1	I
Noetiidae	*Arcopsis ornata* (Viader, 1951)	+/+	1	0	0	1	0	1	0	IO
Glycymerididae	*Glycymeris laddi* (Abrard, 1946)	+/+	1	0	0	0	0	1	?	?
	Tucetona fijiensis (Ladd, 1934)	+/+	0	1	0	0	0	1	?	?
Philobryidae	*Cratis ?kanekoi* Hayami and Kase, 1993	+/+	1	0	0	1	1	1	0	IO
Mytilidae	*Botula fusca* (Gmelin, 1791)	+/+	1	0	0	1	0	1	1	IO
	Lithophaga sp(p).	-/-	1	1	0	?	?	?	?	?
	?Fungiacava sp.	-/-	0	0	1	?	?	?	?	?
	Modiolus ?auriculatus Krauss, 1848	+/+	0	0	1	1	1	1	1	I
	?Septifer sp.	-/-	1	0	0	?	?	?	?	?
	?Mytilidae n. gen. sp. 1	+/+	1	0	0	0	0	?	?	?
Pinnidae	Pinnidae sp.	-/-	1	0	0	?	?	?	?	?
Ostreidae	Ostreidae sp. 1	+/+	1	0	0	1	0	1	0	I
Anomiidae	*Anomia* sp.	+/-	1	0	0	?	?	?	?	?
Pteriidae	*Pinctada* sp.	-/-	1	0	0	?	?	?	?	?
Isognomonidae	*Isognomon* sp.	+/-	0	0	1	?	?	?	?	?
Limidae	*Lima vulgaris* (Link, 1807)	+/+	1?	1	1	1	0	1	1	IO
	Limaria ?fragilis (Gmelin, 1791)	+/+	1	0	0	1	1	1	1	IO
	Ctenoides annulatus (Lamarck, 1819)	+/+	1	0	0	1	1	1	0	IO
Plicatulidae	*Plicatula* sp(p).	+/-	1	0	1	?	?	?	?	?
Pectinidae	*Gloripallium* sp. 1	+/+	1	0	0	0	0	?	?	?
	G. pallium (Linné, 1758)	+/+	1	0	1	1	1	1	1	IO
	Laevichlamys irregularis (Sowerby, 1842)	+/+	1	0	0	1	1	1	1	IO
	L. aff. *squamosa* (Gmelin, 1791)	+/+	1	0	0	0	0	?	?	?
	Semipallium tigris (Lamarck, 1819)	+/+	1	0	1	1	1	1	1	IO
	S. amicum (Smith, 1885)	+/+	0	0	1	1	1	1	0	O
	Excellichlamys aff. *spectabilis* (Reeve, 1853)	+/+	1	0	0	0	0	?	?	?
	Mirapecten rastellum (Lamarck, 1819)	+/+	0	0	1	1	1	1	1	O
Spondylidae	*Spondylus violacescens* Lamarck, 1819	+/+	0	0	1	1	1	1	1	IO
	S. sp(p).	+/-	1	1	1	?	?	?	?	?
Lucinidae	*Codakia punctata* (Linné, 1758)	+/+	1	0	0	1	1	1	1	IO
	C. tigerina (Linne, 1758)	+/+	1	0	0	1	1	1	1	IO
	"Ctena" bella (Conrad, 1837)	+/+	1	0	1	1	1	1	1	IO
	Wallucina aff. *haddoni* (Melvill and Standen, 1899)	+/+	1	0	0	0	0	?	?	?
	W. sp. 4	+/-	1	0	0	?	0	?	?	?
	Pillucina aff. *spaldingi* (Pilsbry, 1921)	+/+	1	0	0	0	0	?	?	?
	Anodontia edentula (Linné, 1758)	+/+	1	0	1	1	1	1	1	IO
	"Parvilucina" sp. 1	+/-	1	0	0	?	0	?	?	?
	"P." sp. 2	+/-	1	0	0	?	0	?	?	?
	Bellucina sp. 1	+/-	1	0	0	?	0	?	?	?

(continued)

Table 1. (continued)

Family	Species	ID	Core Lagoon	Core? Fore reef	Marginal Fore reef	Globally Extant?	Locally Extant?	Range to W	Range to E	Habitat
Fimbriidae	*Fimbria* sp. 1	+/+	1	0	0	0	0	?	?	?
	F. sp. 2	+/+	0	1	0	0	0	?	?	?
Chamidae	*Chama lazarus* Linné, 1758	+/+	1	0	0	1	0	1	0	I
	C. asperella Lamarck, 1819	+/+	1	0	1	1	1	1	1	IO
	C. iostoma Conrad, 1837	+/+	0	0	1	1	1	1	1	IO
	C. sp. 4	+/+	0	0	1	1	1	1	0	O
	C. sp. 7	+/+	1	0	0	0?	0	?	?	?
Carditidae	*Cardita variegata* Bruguiére, 1792	+/+	1	0	0	1	0	1	1	I?
Cardiidae	*Vasticardium* n. sp. (Vidal, pers. comm.)	+/+	1	0	0	1	0	1	0	?
	V. orbita philippinense (Hedley, 1899)	+/+	1	0	1	1	1	1	0	IO
	Acrosterigma sp. 1	+/+	1	0	0	0	0	?	?	?
	Ctenocardia aff. *fornicata* (Sowerby, 1841)	+/+	0	1	0	0	0	?	?	?
	Microfragum ?festivum (Deshayes, 1855)	+/+	0	0	1	1	1	1	0	O
	Fragum sp. 1	+/+	1	0	0	0	0	?	?	?
	F. cf. *fragum* (Linné, 1758)	+/+	1	0	0	1	0	1	1	I
	Corculum sp. 1	+/+	1	0	0	0	0	?	?	?
	C. aff. *cardissa* (Linné, 1758)	+/+	1	0	0	0	0	?	?	?
	Hippopus hippopus (Linné, 1758)	+/+	1	0	0	1	0	1	0	I
	Tridacna maxima (Röding, 1798)	+/+	1	0	1	1	1	1	1	IO
	T. squamosa Lamarck, 1819	+/+	0	0	1	1	1	1	1	IO
	T. derasa (Röding, 1798)	+/+	1	0	0	1	0	1	0	I?
	T. gigas (Linné, 1758)	+/+	1	0	0	1	0	1	0	I?
Tellinidae	*Tellina* aff. *crucigera* Lamarck, 1818	+/+	1	0	0	0	0	?	?	?
	T. chariessa Salisbury, 1934	+/+	1	0	0	1	0	1	0	I?
	T. remies Linné, 1758	+/+	1	0	0	1	0	1	0	I?
	T. scobinata Linné, 1758	+/+	1	0	0	1	1	1	1	IO
	T. gargadia auctt. *non* Linné, 1758	+/+	1	0	0	1	0	1	0	I?
	T. bougei Sowerby, 1909	+/+	1	0	0	1	1	1	1	IO
	T. robusta Hanley, 1844	+/+	1	0	0	1	0	1	1	I
	T. sp. 1	+/+	1	0	0	0	0	?	?	?
	T. (Pinguitellina) sp. 1	+/+	1	0	0	0	0	?	?	?
	T. semen Hanley, 1845	+/+	1	0	0	1	0	1	1	IO
	Loxoglypta clathrata (Deshayes, 1835)	+/+	1	0	0	1	1	1	1	IO
	Scissulina dispar (Conrad, 1837)	+/+	1	0	0	1	0	1	1	I
	Macominae sp. 1	+/-	1	0	0	?	0	?	?	?
Semelidae	*Semelangulus crebrimaculatus* (Sowerby, 1868)	+/+	1	0	1	1	1	1	1	IO
	S. sp. 3	+/+	0	0	1	1	1	1	0	IO
	Leptomya psittacus Hanley, 1882	+/+	1	0	0	1	0	1	0	I
	Ervilia bisculpta Gould, 1861	+/+	1	0	0	1	0	1	1	IO
Psammobiidae	*Gari squamosa* (Lamarck, 1818)	+/+	1	0	0	1	0	1	0	?
	G. pusilla Bertin, 1880	+/+	1	0	0	1	0	1	0	?
Mesodesmatidae	*Atactodea striata* (Gmelin, 1791)	+/+	1	0	0	1	0	1	0	I?
Trapezidae	*Coralliophaga coralliophaga* (Gmelin, 1791)	+/+	1	0	0	1	0	1	1	?
	Trapezium oblongum (Linné, 1758)	+/+	1	0	1	1	1	1	1	IO
	Glossocardia ?stoliczkana Prashad, 1932	+/+	0	1	0	1	0	1	0	?
	G. obesa (Reeve, 1843)	+/+	1	0	0	1	0	1	1	I
Veneridae	*Globivenus toreuma* (Gould, 1850)	+/+	0	1	1	1	1	1	1	IO
	Periglypta reticulata (Linné, 1758)	+/+	0	0	1	1	1	1	1	IO
	Lioconcha ornata (Dillwyn, 1817)	+/+	1	0	0	1	1	1	1	IO
	Pitar sp. 7	+/+	1	0	0	0	0	?	?	?
	Timoclea cf. *marica* (Linné, 1758)	+/+	1	0	0	1	0	1	0	I
	Gafrarium pectinatum (Linné, 1758)	+/+	1	0	0	1	0	1	1	I
	G. sp. 1	+/+	1	0	0	0	0	?	?	?

Niue today includes none of the species of the Pliocene lagoon (Fig. 4). Most (77%) of the locally extinct species in the Pliocene lagoon fauna are inner-reef specialists.

In contrast, among outer-reef inhabitants, only 22% (six of 27 species) suffered local extinction (Fig. 4). Further, three (*Tellina semen, Arcopsis ornata, Ervilia bisculpta*) of the six species not found living on Niue are micromollusks, which I sampled less thoroughly in the Recent than in the Pliocene biota. Thus, the data indicate that the ranges of species whose habitat is not drastically altered by sea-level fluctuations exhibit long-term stability.

Similar distributional stability is exhibited by the fauna of the Pleistocene fore reefs deposited onto the atoll core around the periphery of Niue. These marginal reef deposits formed after initiation of island uplift, and represent habitats comparable to those on today's outer reef. Thus, no major change in available habitats occurred between the time of these Pleistocene faunas and today, a time spanning at least one full sea-level cycle. Only two (8%, N = 26) of the identified bivalves encountered in these peripheral deposits were not encountered in the Recent fauna: *Lima vulgaris* and *Barbatia amygdalumtostum.* The former species is ubiquitous in southern Polynesia today, but was not found living on Niue. The latter species today ranges no further east than Tonga and Samoa, however, the fossils indicate that during at least a brief period in the Pleistocene, *B. amygdalumtostum* extended as far east as Niue. Among Recent bivalve species on Niue, two appear to be restricted to western Pacific islands today; these two occur in Niue's Pleistocene reef deposits, indicating some long-term stability to their ranges. One of the two, *Semipallium amicum*, is common today on Niue and is further known from Palau and Tonga (T. R. Waller, pers. comm.). The other species, *Barbatia* sp. 1 (apparently undescribed), is presently abundant on Niue, and also occurs on eastern Fiji, Wallis Island, and Palmerston Atoll.

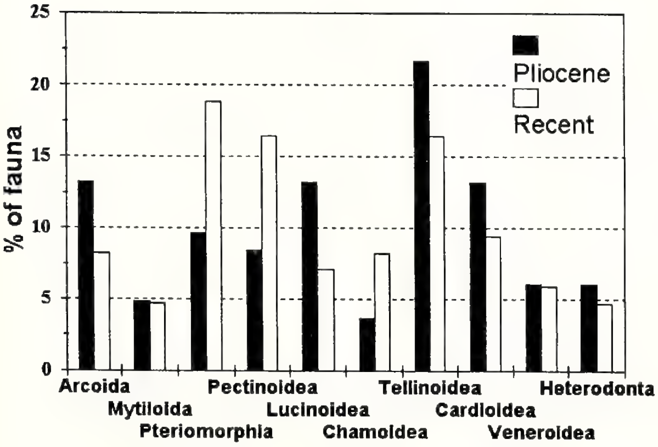

Fig. 2. Comparison of diversity of suprafamilial taxa between Pliocene lagoonal fauna (N = 83 species) and Recent fauna (N = 85 species) of Niue. Pteriomorphia = other than Arcoida, Mytiloida, and Pectinoidea; Heterodonta = other than Lucinoidea, Chamoidea, Tellinoidea, Cardioidea, and Veneroidea, includes two myoids in Recent fauna.

Table 2. Comparison of per-family species richness between Pliocene lagoonal fauna and Recent fauna of Niue Island. Number of species given for each family under each fauna.

Family	Pliocene	Recent
Arcidae	8	6
Noetiidae	1	0
Glycymerididae	1	0
Philobryidae	1	1
Mytilidae	4	4
Pinnidae	1	2
Ostreidae	1	0
Gryphaeidae	0	1
Anomiidae	1	1
Dimyidae	0	1
Pteriidae	1	2
Isognomonidae	0	2
Malleidae	0	1
Limidae	3	5
Plicatulidae	1	1
Propeamussiidae	0	1
Pectinidae	6	9
Spondylidae	1	4
Lucinidae	10	6
Fimbriidae	1	0
Chamidae	3	7
Carditidae	1	0
Cardiidae	11	8
Tellinidae	13	6
Semelidae	3	6
Psammobiidae	2	2
Mesodesmatidae	1	0
Trapezidae	3	1
Glossidae	0	1
Veneridae	5	5
Corbulidae	0	1
Gastrochaenidae	0	1

GEOGRAPHIC RANGE CONTRACTIONS

A corollary to the observation that inner-reef specialists undergo extinction in the central Pacific during regressions is that lagoonal faunas in the area are reassembled anew during each high sea stand. Given the limited time available during each high sea stand and the somewhat stochastic nature of dispersal, the geographic ranges of species might be different at different high sea stands. There is evidence for such range differences in Niue's fossil fauna (Paulay, unpub.), as well as in the fossil record of central Pacific bivalves. With regard to the latter, in surveys of the fossil bivalve faunas of Polynesian and Micronesian islands, I have encountered four species that are each known as fossils on two or more islands on which they are not extant. The fact that these range contractions involve more than one island supports the hypothesis that

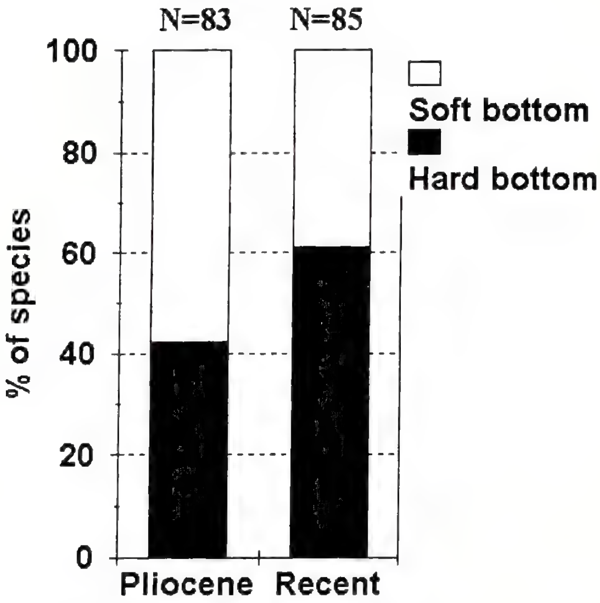

Fig. 3. Comparison of proportions of soft-bottom versus hard-bottom species between Pliocene lagoonal fauna and Recent fauna of Niue. Proportions significantly different between the two faunas (G-test, $p < 0.05$).

regional rather than local processes were responsible for the local extinctions. All four species - *Septifer excisus* (Wiegmann, 1837), *Anadara antiquata* (Linné, 1758), *Tridacna gigas*, and *Hippopus hippopus* - are largely or entirely restricted to inner reefs, as discussed below.

The mussel *Septifer excisus* forms large aggregations on intertidal reef flats in the Mariana Islands, although occasional individuals are also found in cryptic habitats on the fore reef. Its present range extends from East Africa and the Red Sea to the Philippine and Mariana Islands (Fig. 5). While the species does not appear to reach the Pacific Plate today, it reached the most isolated islands of the Pacific Plate during the Pleistocene. It was common in the Hawaiian Islands in shallow water Pleistocene communities (Kosuge, 1969; as *Septifer vaughani* Dall, Bartsch and Rehder, 1938, a synonym, Paulay, 1996), was locally abundant in the shallowest late Pleistocene apron reefs of Mangaia (Cook Island), and was occasional in the Pleistocene lagoon of Henderson Island (Pitcairn Group) (Paulay, 1996).

Anadara antiquata and *A. uropigimelana* are two species in the genus that inhabit islands of the Pacific Plate; both appear to be inner-reef specialists (Paulay, 1990 - both listed as *A. antiquata*). *A. antiquata* extends from East Africa and the Red Sea (Kilburn, 1983) to at least the Mariana Islands and Fiji, with fossil specimens known from the Hawaiian Islands (Kay, 1979) and Aitutaki (Cook Island; Paulay, unpub.). *A. uropigimelana* extends from East Africa (Kilburn, 1983) to the Society Islands and shows local extinction on Niue.

The giant clams *Tridacna gigas* and *Hippopus hippopus* are also largely restricted to inner reefs. *T. gigas* can be common in shallow lagoonal areas, but is very rare on the steep slopes of outer reefs. The species is presently restricted to the western Pacific (Fig. 6). Its range contracted slightly during the Holocene due to apparently human-caused extirpations in the Carolines (Yap: Price and Fagolimul, 1988; Kosrae: pers. obs.) and Fiji (Lewis *et al.*, 1988). However, *T. gigas* suffered range contraction even prior to the arrival of humans: Plio-Pleistocene but no Holocene fossils are known from Guam (Rosewater, 1965; H. G. Siegrist, pers. comm.), Aldabra (Taylor, 1978), Kenya (Crame, 1986), and Niue.

Hippopus hippopus is the giant clam most limited in its depth tolerance: it is restricted to very shallow (< 3 m) reef flats and moats, and is absent from outer reef slopes (R. Rowan, pers. comm.). Its shallow zonation makes this species especially vulnerable to human predation as well as to sea-level fluctuations, both of which appear to have contributed to limiting the present distribution of the species. Today, *H. hippopus* is confined to the western Pacific from western Indonesia to the Marshall Islands and Tonga (Fig. 7). The occurrence of Holocene subfossils but absence of living animals on Guam (B. D. Smith, pers. comm.; pers. obs.) and Fiji (Lewis *et al.*, 1988), and the possible recent

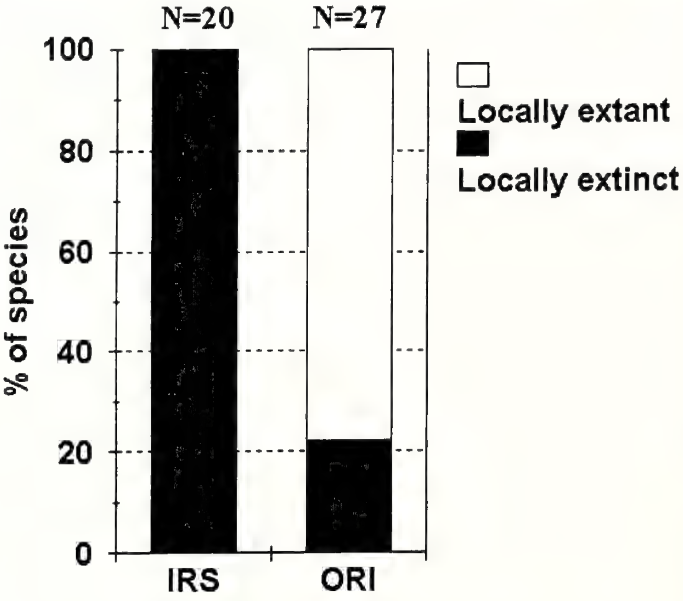

Fig. 4. Comparison of frequency of local extinction among inner-reef specialist (IRS) species versus among outer-reef inhabitant (ORI) species. Proportions significantly different between the two groups (G-test, $p < 0.001$). Only globally extant, Pliocene lagoonal species with known habitat specificity are considered.

extinction in Tonga (Lewis *et al.*, 1988), likely reflect human predation. However, the species also experienced pre-Holocene range contractions at both its eastern (Niue) and western (Aldabra: Taylor, 1978) distributional boundary, probably due to sea-level fluctuations.

The above distributional changes of inner-reef bivalve species supports the hypothesis that the ranges of inner-reef specialists in the central Pacific differ between different high sea stands. The significant range contractions noted in several species since the Plio-Pleistocene demonstrate that at least some of the endemic diversity of the western Pacific is the result of the area being a sink for species that have been extirpated elsewhere. Springer (1982) argued that most taxa that presently do not extend onto the Pacific Plate were always so delimited. Niuean fossils and the species noted above include at least four species that are known as fossils on the Pacific Plate but no longer occur there: *Septifer excisus* (above), *Tridacna derasa* (with an eastern distributional limit along the western Pacific island arcs), *Glossocardia* ? *stoliczkana* (known extant from Indonesia and the Philippines only; A. Matsukuma, pers. comm.), and *Vasticardium* n. sp. (known from Moluccas, Papua New Guinea, Admiralty Islands, and Solomons only; J. Vidal, pers. comm.).

LONG TERM CONSEQUENCES

The range contractions and expansions discussed here occur in association with Milankovitch cycles. These cycles have a periodicity (20-100 Ma) that lies between ecological timescales, during which typical population processes operate, and geological timescales, over which much of macroevolutionary change occurs (Bennett, 1990). What are the long-term effects of the recurrent regional extinctions and resulting range contractions suffered by inner-reef specialists? There are a number of hypothesized consequences: (1) a higher global extinction rate among inner-reef specialists than among outer-reef inhabitants, whose metapopulations exhibit relative long-term stability; (2) facilitation of speciation among inner-reef specialists, as regression-caused extinction of intermediate populations divides previously contiguous species ranges (*e.g.* Fleminger, 1986; Springer and Williams, 1990); and conversely, (3) inhibition of speciation among inner-reef specialists, as regression-caused extinction on isolated islands precludes the divergence of such populations into island endemics.

The limited evidence available on Niue does not support the hypothesis of higher rates of global extinction among inner-reef specialists than among outer-reef inhabitants. A limitation in testing this hypothesis, however, is that the habitat specificities (with regard to inner versus outer reef) of extinct species cannot be reliably determined. To partially circumvent this problem, I can use one of two alternative proxies - recognizing, however, that these substitute comparisons could fail to detect existing differences in extinction rates. One such proxy compares extinction rates between lagoonal and fore-reef assemblages: lagoonal assemblages have a large proportion (43% among extant species of the Pliocene fauna) of inner-reef specialists, while fore-reef assemblages lack inner-reef specialists

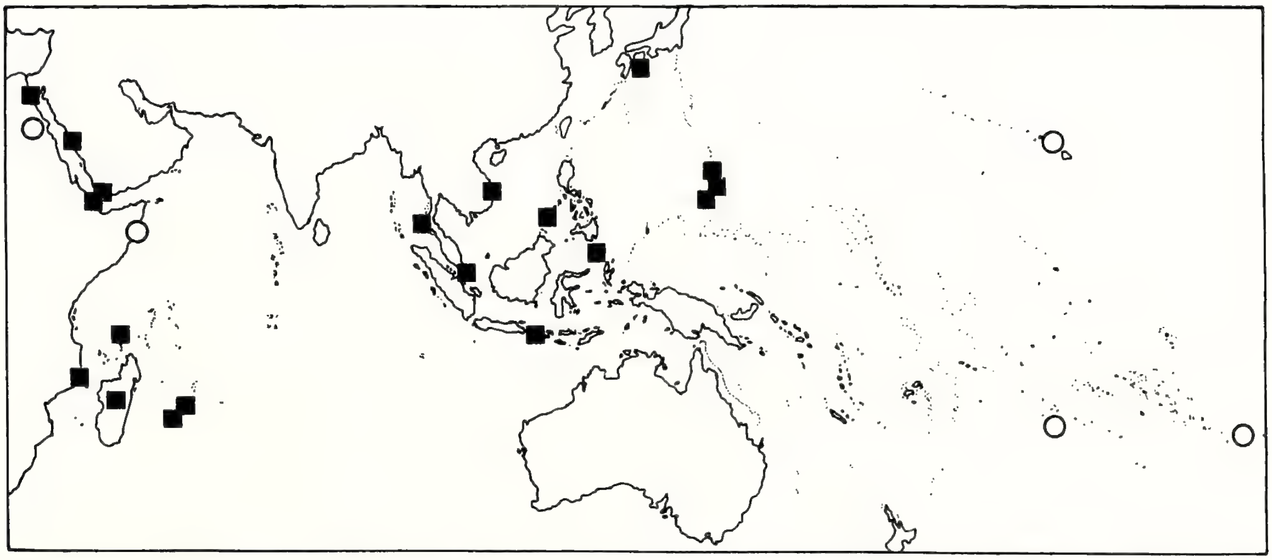

Fig. 5. Present and past distribution of *Septifer excisus*. Based on USNM and Museum of Comparative Zoology records (see Paulay, 1996), and literature records from: Newton (1900), Dautzenberg (1929), Prashad (1932), Lamy (1937), and Abrard (1940). (Squares, extant records; empty circles, Pleistocene records).

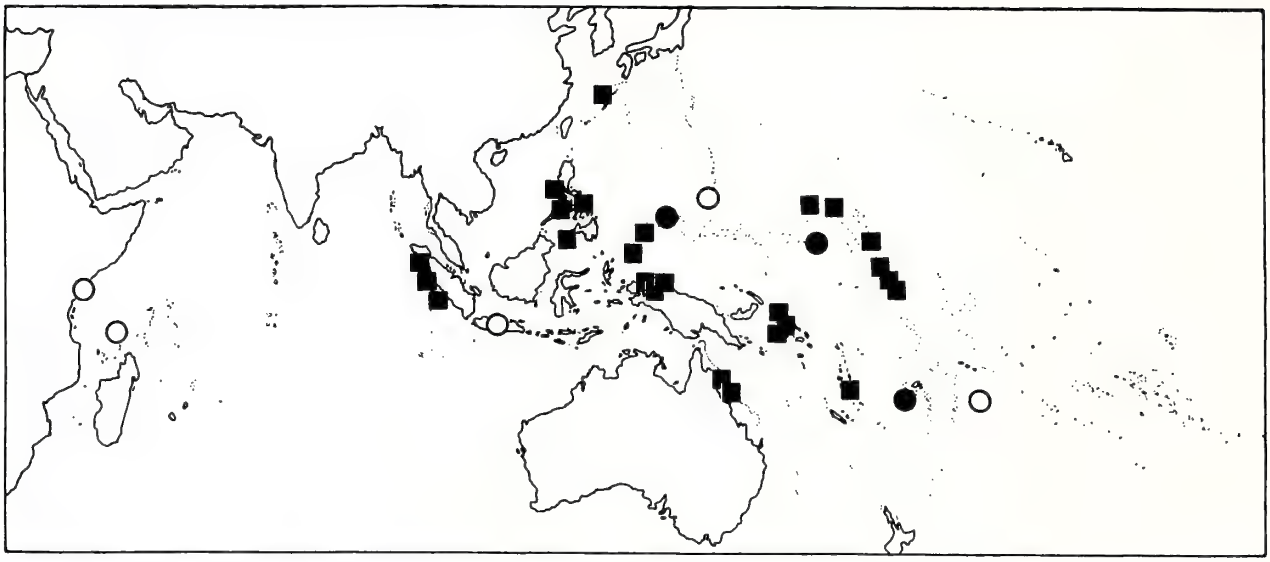

Fig. 6. Present and past distribution of *Tridacna gigas.* Based on literature records from Rosewater (1965), Taylor (1978), Hirschberger (1980), Crame (1986), Lewis *et al.* (1988), Price and Fagolimul (1988), and additional data herein, including additional Marshall and Tungaru records, and Kosrae Holocene record. [Squares, extant records; solid circles, Holocene records, but presumed extinct; empty circles, Plio-Pleistocene records (and Miocene from Java)].

(transport of dead shells from lagoons to fore reefs appears to be negligible on central Pacific islands; Paulay, 1990). Seven identified species are known from putative core fore-reef deposits on Niue (Table 1; see above), and 43% of these are extinct. Among species in the lagoonal assemblage, 28% have gone extinct. These extinction rates are in the opposite direction than predicted.

An alternative proxy compares the proportion of species that were *likely* inner-reef specialists among the extinct portion of the Pliocene fauna with the proportion *known* to be inner-reef specialists among the extant portion of the Pliocene fauna. Three of the extinct species are known from fore-reef deposits and are thus definitely outer-reef inhabitants. Four others belong to genera *(Gafrarium, Fragum, Corculum)* whose extant species in the Central Pacific are all inner-reef specialists; thus their extinct counterparts were likely also inner-reef specialists. Based on these seven extinct species, the Pliocene fauna (here including both fore-reef and lagoonal deposits) included comparable proportions of inner-reef specialists among its extinct (57%) and extant (43%, N = 47) species.

Sea level fluctuations can be important agents of vicariance, separating once-continuous species ranges by (1) forming terrestrial or oceanographic barriers (*e.g.* Fleminger, 1986) or (2) causing the extinction of intermediate populations (*e. g.* Springer and Williams, 1990). The first of these processes is possibly exemplified in two areas of the Indo-West Pacific (Rosen, 1984): during maximal glacial low-sea stands, emergent land isolated the Red Sea from the western Indian Ocean, and greatly constricted the Indo-Malayan connection between eastern Indian and western Pacific Oceans (Rosen, 1984; Fleminger, 1986; Sheppard and Sheppard, 1991). Thus, species richness in these two areas could be in part the product of a sea-level-driven diversity pump (Rosen, 1984). Additional, smaller-scale vicariant separations could have occurred within the complex maze of islands and basins in Indo-Malaya (McManus, 1985).

Although no such regression-associated land barriers are apparent in the insular Pacific, vicariance by extinction of intermediate populations is conceivable and could be driven by sea-level fluctuations. If inner-reef specialists find habitat-related refugia in widely separated areas, then regressions will lead to the fragmentation of their ranges. Such refugia are most likely in the western Pacific (Melanesia, Indo-Malaya, etc.) and on a few, unusual central Pacific islands with reef systems that are minimally disrupted by regressions (*e. g.* Marquesas, Rapa; Paulay, 1990). The relatively high proportion of endemics in the Marquesan and Rapan faunas (Rehder, 1968; Richard, 1984; Randall, 1985) could reflect this process of speciation.

Numerous species that are distributed widely among the islands of Polynesia and Micronesia are nonetheless restricted to the Pacific Plate. To explain the apparent paradox of these widespread Pacific Plate endemics, Springer and Williams (1990) invoked vicariance. Observing that the sister species of several Pacific Plate endemics are restricted to the Indian Ocean, the authors proposed that there previously existed intermediate populations - in the

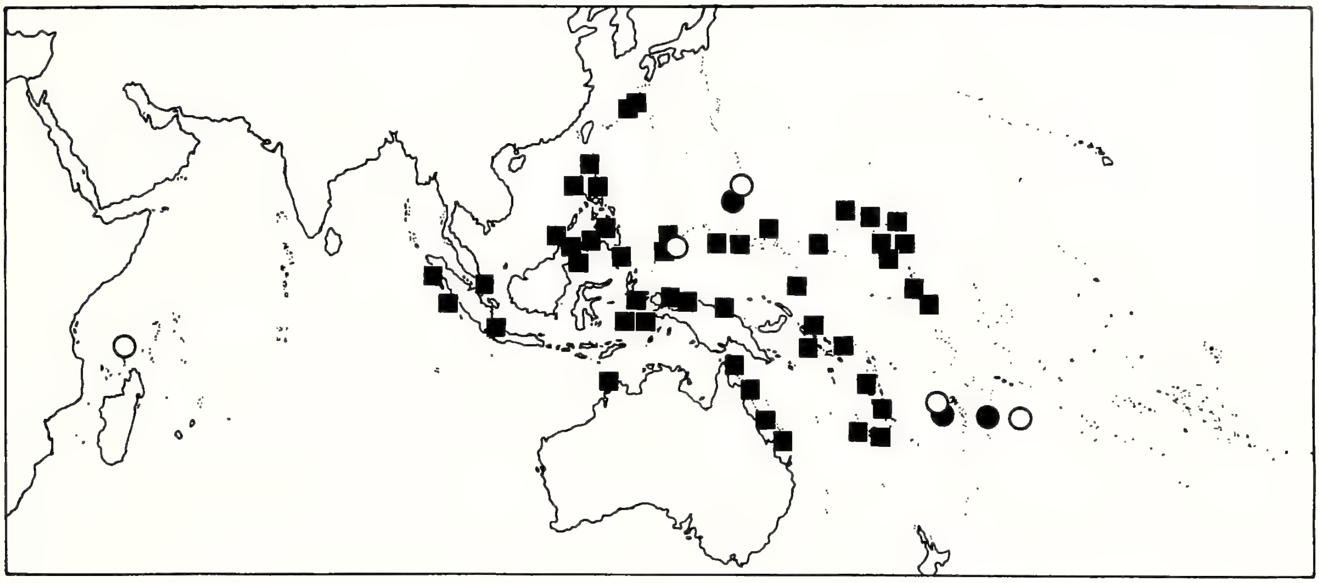

Fig. 7. Present and past distribution of *Hippopus hippopus*. Based on this study and literature records from Rosewater (1965), Taylor (1978), and Lewis *et al.* (1988). (Squares, extant records; solid circles, Holocene records, but presumed extinct; empty circles, Plio-Pleistocene records).

western Pacific Ocean - that have since gone extinct. They hypothesized that the extinction was caused by cooling through increased upwelling and by regression-caused emergence in the area. Indeed, habitat loss due to glacial regressions was high on the large continental shelves of western Indonesia. However, other parts of the western Pacific (*e. g.* the Philippines, Moluccas, Melanesia) were no more affected by habitat loss than central Pacific islands (Myers, 1989), and in fact likely served as habitat refuges for numerous inner-reef-specialist species (Paulay, 1990). Further, although areas of Indo-Malaya could have experienced cooling during glacial times due to increased rates of upwelling (Fleminger, 1986), there is no evidence that Melanesia or the Philippine region were similarly affected. Thus the absence of widespread Pacific endemics from areas like Melanesia and the Philippines is not well-explained by Springer and Williams' (1990) hypothesis.

A purely ecological hypothesis might best explain the phenomenon of widespread Pacific Plate endemics. Organisms that require oceanic marine conditions will thrive in the central Pacific but might not survive in the western Pacific, where large islands - with substantial terrigenous sedimentary and nutrient input - predominate. Abbott (1960) and George (1974) recognized that a significant number of species in the Indo-West Pacific are restricted to areas that are oceanic in character (and others to areas that are continental). Ecological restriction to oceanic conditions could explain not only widespread Pacific endemics, but also other taxa that exhibit disjunct distributions between oceanic areas of the Pacific and Indian Oceans. A similar ecological hypothesis has been invoked to explain why certain land crab and sea bird species occur to the west and east of, but not in, Indo-Malaya: mammalian predators, absent from most oceanic islands, prevent these species from establishing in the continental area (Indo-Malaya) (Hartnoll, 1988; Steadman, 1989; Paulay, 1994).

Finally, sea-level fluctuations could influence the likelihood of speciation on isolated Pacific islands by affecting the persistence of insular populations. The short-lived populations of inner-reef specialists might not have sufficient time to differentiate into endemics, especially when compared to the long-lived populations of outer-reef inhabitants. I have previously shown (Paulay, 1990) that endemics are significantly more common among outer-reef inhabitants than among inner-reef specialists on central Pacific islands. A corollary to this hypothesis is that inter-island, intraspecific geographic differentiation is expected to be more prevalent and of greater magnitude among outer-reef inhabitants than among inner-reef specialists. No comprehensive database is presently available to test these ideas, but the prevalence of geographic variation in outerreef inhabitants is striking. Examples include species/subspecies complexes of the turbinid *Astralium rhodostoma* (Lamarck, 1822), the mytilids *Septifer cumingi* Récluz, 1849, and *Septifer furcillata* Gould, 1861, the cardiid *Vasticardium orbita* (Broderip and Sowerby, 1833), the limid *Lima vulgaris,* and the venerid *Dorisca cookei* Dall, Bartsch and Rehder, 1938 (Paulay, unpub.; J. Vidal, pers. comm.).

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LITERATURE CITED

Abbott, R. T. 1960. The genus *Strombus* in the Indo-Pacific. *Indo-Pacific Mollusca* 1:33-146.

Abrard, R. 1940. Mollusques Pleistocenes de la côte française des Somalis, recueillis par E. Aubert de la Rue. *Archives du Muséum National d'Histoire Naturelle, 6e serié* 18:1-105.

Aharon, P., S. L. Goldstein, C. W. Wheeler, and G. Jacobson. 1993. Sea-level events in the South Pacific linked with the Messinian salinity crisis. *Geology* 21:771-775.

Bennett, K. D. 1990. Milankovitch cycles and their effects on species in ecological and evolutionary time. *Paleobiology* 16:11- 21.

Boss, K. J. 1982. Mollusca. *In: Synopsis and Classification of Living Organisms,* S. P. Parker, ed. pp. 945-1166. McGraw-Hill, New York.

Cernohorsky, W. O. 1970. The littoral marine molluscs of Niue Island. *Records of the Auckland Institute and Museum, 3rd series* 7:175-186.

Chevalier, J. P. 1979. La faune corallienne (Scleractiniaires et Hydrocoralliaires) de la Polynésie française. *Cahiers de l'Indo-Pacifique* 1:129-151.

Crame, J. A. 1986. Late Pleistocene molluscan assemblages from the coral reefs of the Kenya coast. *Coral Reefs* 4:183-196.

Darwin, C. 1842. *The Structure and Distribution of Coral Reefs.* Smith, Elder, London. 214 pp.

Dautzenberg, P. 1929. Mollusques testacés marins de Madagascar. *Faune des Colonies Françaises, Société d'Editions* 3:321-636.

Davis, M. B. 1981. Quaternary history and stability of forest communities. *In: Forest Succession: Concepts and Applications,* D. C. West, H. H. Shugart, and D. B. Botkin, eds. pp. 132-153. Springer-Verlag, New York.

Dubois J., J. Launay, and J. Recy. 1975. Some new evidence on lithospheric bulges close to island arcs. *Tectonophysics* 26:189-196.

Fleminger, A. 1986. The Pleistocene equatorial barrier between the Indian and Pacific Oceans and a likely cause for Wallace's line. *In: Pelagic Biogeography,* A. C. Pierrot-Bults, S. van der Spoel, B. J. Zahuranec, and R. K. Johnson, eds. pp. 84-97. UNESCO Technical Papers in Marine Science 49.

George, R. W. 1974. Coral reefs and rock lobster ecology in the Indo-West Pacific region. *Proceedings of the Second International Coral Reef Symposium, Brisbane* 1:321-325.

Grigg, R. W. 1982. Darwin point: a threshold for atoll formation. *Coral Reefs* 1:29-34.

Hartnoll, R. G. 1988. Evolution, systematics, and geographical distribution. *In: Biology of Land Crabs,* W. W. Burggren and B. R. McMahon, eds. pp. 6-54. Cambridge University Press, Cambridge.

Hirschberger, W. 1980. Tridacnid stocks on Helen Reef, Palau, western Caroline Islands. *Marine Fisheries Review* Feb. 1980:8-15.

Kay, E. A. 1979. *Hawaiian Marine Shells. Reef and Shore Fauna of Hawaii. Section 4: Mollusca.* Bishop Museum Press, Honolulu. xviii + 653 pp.

Kilburn, R. N. 1983. The Recent Arcidae (Mollusca: Bivalvia) of southern Africa and Mozambique. *Annals of the Natal Museum* 25:511-548.

Kosuge, S. 1969. Fossil mollusks from Oahu, Hawaii Islands. *Bulletin of the National Science Museum, Tokyo* 12:783-794.

Lamy, E. 1937. Révision des Mytilidae vivants du Muséum National d'Histoire Naturelle de Paris. *Journal de Conchyliologie* 81:99-132, 169-197.

Lewis, A. D., T. J. H. Adams, and E. Ledua. 1988. Fiji's giant clam stocks - a review of their distribution, abundance, exploitation and management. *In: Giant Clams in Asia and the Pacific,* J. W. Copland and J. S. Lucas, eds. pp. 66-72. Australian Centre for International Agricultural Research, Canberra.

McManus, J. W. 1985. Marine speciation, tectonics and sea-level changes in southeast Asia. *Proceedings of the Fifth International Coral Reef Congress, Tahiti* 4:133-138.

McNutt, M. and H. W. Menard. 1978. Lithospheric flexure and uplifted atolls. *Journal of Geophysical Research* 83:1206-1212.

Montaggioni, L. F. 1985. Makatea Island, Tuamotu Archipelago. French Polynesian coral reefs: reef knowledge and field guides. *Fifth International Coral Reef Congress, Tahiti* 1:103-157.

Myers, R. F. 1989. *Micronesian Reef Fishes.* Coral Graphics, Guam. vi + 298 pp., 144 pls.

Newton, R. B. 1900. Pleistocene shells from the raised beach depsoits of the Red Sea. *Geological Magazine, n. s.* 7:500-514, 544-560.

New Zealand Map Service (NZMS). 1985. *Map of Niue, New Zealand Map Service 250.* Department of Land and Surveys, New Zealand.

Paulay, G. 1987. Biology of Cook Islands' bivalves, Part I. Heterodont families. *Atoll Research Bulletin* 298:1-31.

Paulay, G. 1990. Effects of Late Cenozoic sea-level fluctuations on the bivalve faunas of tropical oceanic islands. *Paleobiology* 16:415-434.

Paulay, G. 1991. Late Cenozoic sea level fluctuations and the diversity and species composition of insular shallow water marine faunas. *In: The Unity of Evolutionary Biology. The Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology,* E. C. Dudley, ed. pp. 184-193. Dioscorides, Portland, Oregon.

Paulay, G. 1994. Biodiversity on oceanic islands: its origin and extinction. *American Zoologist* 34:134-144.

Paulay, G. 1996. New records and synonymies of Hawaiian bivalves. *Occasional Papers of the Bishop Museum* 45:18-29.

Paulay, G. and L. R. McEdward. 1990. A simulation model of island reef morphology: the effects of sea level fluctuations, growth, subsidence and erosion. *Coral Reefs* 9:51-62.

Paulay, G. and T. Spencer. 1988. Geomorphology, palaeoenvironments and faunal turnover, Henderson Island, S.E. Polynesia. *Proceedings of the Sixth International Coral Reef Symposium, Townsville* 3:461-466.

Paulay, G. and T. Spencer. 1992. Niue Island: geologic and faunatic [sic] history of a Pliocene Atoll. *Pacific Science Association Information Bulletin* 44:21-23.

Pirazzoli, P. A. and L. F. Montaggioni. 1985. Lithospheric deformation in French Polynesia (Pacific Ocean) as deduced from Quaternary shorelines. *Proceedings of the Fifth International Coral Reef Congress, Tahiti* 3:195-200.

Prashad, B. 1932. The Lamellibranchia of the Siboga Expedition. Systematic part II Pelecypoda (exclusive of the Pectinidae). *Siboga Expedition Reports, Monograph* 53C:1-353.

Price, C. M. and J. O. Fagolimul. 1988. Reintroduction of giant clams to Yap State, Federated States of Micronesia. *In: Giant Clams in Asia and the Pacific,* J. W. Copland and J. S. Lucas, eds. pp. 41-46. Australian Centre for International Agricultural Research, Canberra.

Randall, J. E. 1985. Fishes. French Polynesian coral reefs: reef knowledge and field guides: fauna and flora; a first compendium of French Polynesian sea-dwellers. *Fifth International Coral Reef Congress, Tahiti* 1:462-481.

Rehder, H. A. 1968. The marine molluscan fauna of the Marquesas Islands. *Annual Reports for 1968 of the American Malacological Union,* pp. 29-32.

Richard, G. 1984. Rapa la méconnue. *Xenophora* 24:7-16.

Rosen, B. R. 1984. Reef coral biogeography and climate through the Late Cainozoic: just islands in the sun or a critical pattern of islands? *In: Fossils and Climate,* P. Brenchley, ed. pp. 201-262. John Wiley and Sons Ltd., New York.

Rosewater, J. 1965. The family Tridacnidae in the Indo-Pacific. *Indo-Pacific Mollusca* 1:347-396.

Salvat, B. 1967. Importance de la faune malacologique dans les atolls polynésiens. *Cahiers Pacifique* 11:7-49.

Schofield, J. C. 1959. The geology and hydrology of Niue Island, South Pacific. *New Zealand Geological Survey Bulletin, n.s.* 62: 28 pp.

Sheppard, C. R. C. and A. L. S. Sheppard. 1991. *Corals and Coral Communities of Saudi Arabia.* Fauna of Saudi Arabia 12. 170 pp.

Simpson, G. G. 1953. *The Major Features of Evolution.* Columbia University Press, New York. xx + 434 pp.

Spencer, T. and G. Paulay. 1989. Geology and geomorphology of Henderson Island. *Atoll Research Bulletin* 323:1-50.

Spencer, T., D. R. Stoddart, and C. D. Woodroffe. 1987. Island uplift and lithospheric flexure: observations and cautions from the South Pacific. *Zeitschrift für Geomorphologie, N. F., Supplement-Band* 63:87-102.

Springer, V. G. 1982. Pacific plate biogeography, with special reference to shorefishes. *Smithsonian Contributions to Zoology* 367:iv + 182 pp.

Springer, V. G. and J. T. Williams. 1990. Widely distributed Pacific plate endemics and lowered sea-level. *Bulletin of Marine Science* 47:631-640.

Stanley, S. M. 1979. *Macroevolution.* W. H. Freeman, San Francisco. xi + 332 pp.

Stanley, S. M., W. O. Addicott, and K. Chinzei. 1980. Lyellian curves in paleontology: possibilities and limitations. *Geology* 8:422-426.

Steadman, D. W. 1989. Extinction of birds in eastern Polynesia: a review of the record, and comparisons with other Pacific island groups. *Journal of Archaeological Science* 16:177-205.

Stoddart, D. R. 1973. Coral reefs: the last two million years. *Geography* 58:313-323.

Taylor, J. D. 1978. Faunal response to the instability of reef habitats: Pleistocene molluscan assemblages of Aldabra atoll. *Palaeontology* 21:1-30.

Waller, T. R. 1990. The evolution of ligament systems in the Bivalvia. *In: The Bivalvia - Proceedings of a Memorial Symposium in Honour of Sir Charles Maurice Yonge, Edinburgh, 1986*, B. Morton, ed. pp. 49-71. Hong Kong University Press, Hong Kong.

Webb, T., III. 1987. The appearance and disappearance of major vegetational assemblages: long-term vegetational dynamics in eastern North America. *Vegetatio* 69:177-187.

Wheeler, C. W. and P. Aharon. 1991. Mid-oceanic carbonate platforms as oceanic dipsticks: examples from the Pacific. *Coral Reefs* 10:101-114.

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Characteristics of submarine cave bivalves in the northwestern Pacific

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Abstract: This paper discusses the diversity, common features, and geographic distrbution of submarine cave bivalves collected with SCUBA from a number of islands around the Philippine Sea (Okinawa, Miyako, Yonaguni, Daito, Bonin, Bohol and Cebu of the Philippines, Palau, and Guam). Common significant characteristics of cave bivalves are: (1) unique taxonomic assemblage, (2) reduced adult size, (3) many deep-water genera, (4) occurrence of several "cavity-dwelling" shallow-water genera on the exposed wall and sediment surface, (5) frequent paedomorphosis by progenesis, (6) relative abundance of non-planktotrophic species, (7) low fecundity and dominance of brooding, and (8) archaic life mode reminiscent of a fauna before the "Mesozoic marine revolution" (rarity of sedentary species and deep burrowers). These features must be related to one another and are generally regarded as due to a common adaptive strategy toward the oligotrophic condition and low predation pressure of cave habitats. It is still mysterious how cave bivalves, even brooding species, have become so extensively distributed in the western Pacific region. Although there is no positive evidence, rafting is a possible mechanism of transoceanic dispersal for minute epibyssate bivalves.

For several years now, we have been studying the cryptic biota of submarine caves with the assistance of several taxonomists and skilled divers. The cave organisms, although their biomass per unit area is very small, include various invertebrate groups such as benthic foraminiferans, sponges, sclerosponges, solitary corals, gorgonians, polychaetes, bryozoans, brachiopods, sipuncules, ostracods, isopods, and echinoids in addition to gastropods and bivalves. Among others, the taxonomic diversity and evolutionary significance of cave mollusks (especially bivalves) were partly elucidated on the basis of numerous samples collected by SCUBA divers from the Ryukyu Islands (Okinawa and Miyako) (Kase and Hayami, 1992; Hayami and Kase, 1992, 1993). More than 45 species have been systematically described by us.

Subsequently, we expanded the area of our study and explored many caves on other islands around the Philippine Sea, namely Yonaguni Island of Ryukyu (one cave), Minami-daito Island (one cave), Chichijima Islands of Bonin (six caves and interior of some sunken ships), Panglao Island of Bohol (three caves), Mactan Island of Cebu (one cave), "Rock Islands" of Palau (six caves), and Guam (two caves) (Fig. 1). Some samples from Guam and Saipan in the synoptic collection of the Marine Laboratory, University of Guam, were also examined. Although living specimens were not necessarily abundant in these newly explored caves, it became clear that many cave bivalves are widely distributed among these distant islands. Up to now, about 60 bivalves have been recognized as cavernicolous. The present paper discusses the common characteristics of cave bivalves as well as their geographic distribution.

SUBMARINE CAVES AROUND THE PHILIPPINE SEA

More than 30 surveyed caves in this region vary in size and topography, but mostly are sublittoral meandering limestone grottoes. Their entrances (Fig. 2) lie between sea level and 40 m, and their lengths range from several meters to more than 70 m. In several relatively flat islets of the Ryukyu Islands numerous caves are open to fore-reef slopes, the mother rock of which is the Pleistocene Ryukyu Limestone. They were undoubtedly formed by underground water during some lower sea-level stage and finally drowned in the post-glacial sea-level rise. If the Pleistocene-Holocene sea-level change in this region is considered, all the marine organisms in these caves must have originated sometime after 10,000 years B.P.

Owing to the low physical energy of the sea water, sediments on the floor of deep caves are generally very fine except for organic remains, and almost free of coarse terrigenous material. Because these caves are already hydrologically inactive, the temperature and salinity of the cave

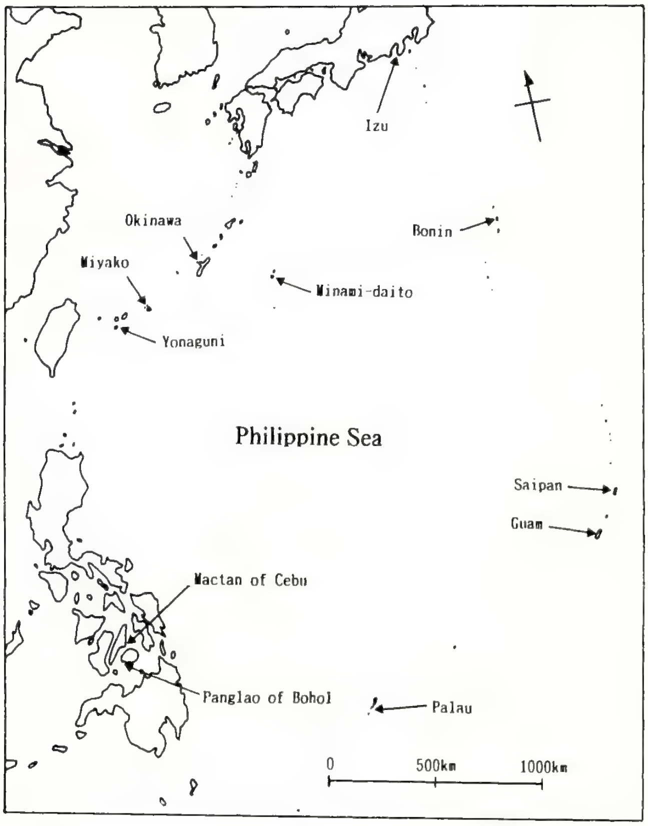

Fig. 1. Map showing the localities of marine cave samples.

waters are almost equal to those of the open sea throughout a year. Tubular sediment cores taken successfully by a cooperative diver at the innermost part of some caves indicate that the environment seems to have been maintained under tranquil but never oxygen-depleted conditions. Many surveyed limestone caves in other islands seem to be basically similar to those of the Ryukyu Islands in physicochemical conditions.

In Okinawa, Yonaguni, Bonin, Bohol, and Palau, the twilight wall and ceiling near the cave entrances are often inhabited by sclerosponge fauna, which consists of sclerosponges, articulate brachiopods, bryozoans, and ahermatypic hexacorals. This cryptic fauna corresponds to "the brachiopod-coralline sponge community," which was recognized by Jackson *et al.* (1971) mainly in the Caribbean Sea. In the present region it is commonly accompanied by *Pycnodonte taniguchii* (a large archaic oyster) (Hayami and Kase, 1992). *Glossocardia obesa* is another exceptionally large bivalve in the cave fauna. In Ryukyu and Palau this clam inhabits poorly lit soft bottom in a number of caves.

Toward the totally dark inner part the biomass becomes much smaller, and the wall and ceiling are rarely covered with encrusting organisms, although *Neritopsis radula* (Linné, 1758) (a well-known "living-fossil" gastropod), *Dimyella* spp. (small characteristic sedentary bivalves), thecidellinid brachiopods, and some soft sponges are often found. Moreover, the sediment surface is inhabited by many minute bivalves and other invertebrates such as benthic foraminiferans, gastropods (mostly archaeogastropods), sipuncules (commonly dwelling in gastropod shells), ostracods, and isopods.

It is a fundamental question whether or not these minute bivalves are really indigenous to such sheltered environments. Because the distribution of such minute bivalves has not been sufficiently studied in this region, it is now difficult for us to give an obvious answer to this question. More than 60 bivalves (Table 1), however, are regarded as cavernicolous (if not strictly indigenous to caves), because they are actually represented by numerous living specimens (or fresh empty shells). The possibility of concentration of immature individuals must be also examined. In many cases, however, asymptotic size distribution indicating adult size and other lines of evidence (*e.g.* parental care of juveniles) seem to deny the possibility of invalid dispersal.

CHARACTERISTIC FEATURES OF CAVE BIVALVES

UNIQUE TAXONOMIC COMPOSITION

The Arcoidea, Limopsoidea, Mytiloidea, Pectinoidea, Limoidea, and Carditoidea are the dominant superfamilies in these caves, not only in species diversity but also in number of individuals. These superfamilies are also common in the exposed environment of the same region, but the familial and generic composition of the cave assemblages are unique. In some caves a species of *Huxleyia* (Nucinelloidea) is also dominant.

The cave Arcoidea consist of several species of *Bentharca* (though atypical) and *Bathyarca*, and the species of the Limopsoidea mostly belong to *Philobrya, Cosa,* and *Cratis* of the Philobryidae. All of the cave Pectinoidea belong to *Parvamussium, Cyclopecten,* and *Chlamydella* of

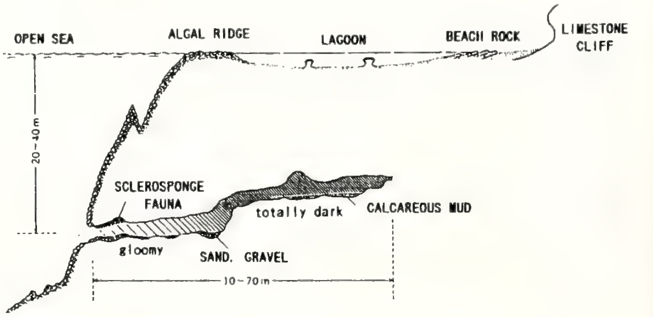

Fig. 2. Diagrammatic section of a submarine cave in the Ryukyu Islands.

Table 1. Distribution and characteristics of cave bivalves in the northwestern Pacific. Bh, Bohol; Bn, Bonin; Dm, Minami-daito; DWG, deep-water genus; Gm, Guam; Hw, Hawaii (not cave); Js, South Japan (not cave); My, Miyako; NP (BR), non-planktotrophic (brooding); Ok, Okinawa; Pl, Palau; PRG, paedomorphic (by progenesis); STD, stunted; Yn, Yonaguni; ●, living; ○, empty shells.

Species name (Hayami and Kase, 1993) (*undescribed species)	Distribution										Characteristics			
	Js	Ok	My	Yn	Ds	Bn	Bh	Pl	Gm	Hw	STD	DWG	PRG	NP(BR)
Solemya (Petrasma) sp.			●											
Huxleyia cavernicola Hayami and Kase, 1993		○	●			●	○	○			+	+	+	+
Pronucula insignis Hayami and Kase, 1993		○	●								+	+	+	+(+?)
P.? sp. *	○					●								
Acar aff. *plicata* (Dillwyn, 1817)		○	○		○	○	○	○			+			
Bentharca tenuis Hayami and Kase, 1993		○	●	○			○	?	?		+	+	+	+
B. decorata Hayami and Kase, 1993		○	○								+	+	+	+(+?)
B. irregularis Hayami and Kase, 1993		○	○				○				+	+	+	
B. excavata Hayami and Kase, 1993		●	●					○			+	+	+	+
B. sp. A *	○					●					+	+	+	
B. sp. B *								○	○		+	+	+	
Bathyarca sp.			○								+	+	+	+
Philobrya sp. *					●								+	+(+?)
Cosa waikikia (Dall, Bartsch and Rehder, 1938)	○	●	●	○		●		○		○			+	+(+)
C. kinjoi Hayami and Kase, 1993		●	●	○			○	○					+	+(+?)
C. uchimae Hayami and Kase, 1993		○											+	+(+?)
C. sp.			○				○						+	+(+?)
Cratis kanekoi Hayami and Kase, 1993	○		○									+	+	+(+?)
Cratis cf. *kanekoi* *									○			+	+	+(+?)
C. ohashii Hayami and Kase, 1993	○	○	○									+	+	+(+?)
Limopsoidea? gen. and sp. indet.		○	○	○			○	○	○					+
Brachidontes sp.		○	○		○									
Septifer sp.			●	○			○	○						
Crenella sp. A		○	●				○				+		+	+
C. sp. B *					○	●					+			
Dacrydium zebra Hayami and Kase, 1993		●	●	○	●	●	○	○				+	+	+(+)
Urumella concava Hayami and Kase, 1993			●	○			○						+	+
Malleus (Malvufundus) sp.		●	●			●					+			
Parvamussium crypticum Hayami and Kase, 1993		●			○						+	+		+
P. cf. *crypticum* *								●			+	+		+
P. decoratum Hayami and Kase, 1993	●		●	○							+	+		+
Cyclopecten ryukyuensis Hayami and Kase, 1993		○	●	○			○	○			+	+		+
Chlamydella incubata Hayami and Kase, 1993	○	○	●			●	○	○	○		+	+		+(+)
C. tenuissima Hayami and Kase, 1993	○	○	●		●	●					+	+		+(+)
Dimyella sp. A *		●					○							
D. sp. B *								○	○					
Pycnodonte taniguchii Hayami and Kase, 1992		●	●	●	○	●	●	●						
Lima sp.		○	●				○	○			+		+	+
Divarilima elegans Hayami and Kase, 1993		○									+	+		+
Ctenoides minimus Hayami and Kase, 1993		○	●		○			○			+		+	+
Isolimea limopsis (Nomura and Zinbo, 1934)	○		○											+
Limatula kinjoi Hayami and Kase, 1993		○	●								+			+(+?)
Limaria sp.		○	●					○						
Epicodakia pygmaea Hayami and Kase, 1993		○	●		○			○			+			
Cardita uruma Hayami and Kase, 1993		●	●								+			
C. sp. A		○									+			
C. sp. B *					○									
Carditella iejimensis Hayami and Kase, 1993		●									+	+		+
C. shimojiensis Hayami and Kase, 1993			●					○			+	+		+
C. sp. *									○					
Condylocardia sp. *						●	○							+(+?)
Salaputium unicum Hayami and Kase, 1993		○	●								+	+		+
S.? sp. *								○						
Rochefortina sandwichensis (Smith, 1885)	○	○	●	○	○	●	○	○		○				
Kelliella japonica Hayami and Kase, 1993		○	○									+		
Coralliophaga hyalina Hayami and Kase, 1993		○	●		○		○	○	○		+		+	
Glossocardia obesa (Reeve, 1843)		●	●		○			●	○					
Exotica sp. *						●								
Irus (Irus) sp.			●				○							
I. (Notirus) sp.		○	●											
Hiatella aff. *orientalis* (Yokoyama, 1920)	○	○	○		○	○	○				+			
Halonympha asiatica Hayami and Kase, 1993		○									+	+		+
Austroneaera sp. *				○				○				+		

the Propeamussiidae (rather than Pectinidae). The occurrence of *Huxleyia, Pronucula, Dacrydium, Divarilima, Carditella, Kelliella,* and *Halonympha* is remarkable, because these genera have been scarcely represented in the upper sublittoral faunas of this region. The discovery of two undescribed species of *Dimyella* is also noteworthy, because this genus was originally proposed for a cave species in the Caribbean region (Moore, 1970). In contrast, the Nuculanoidea, Pinnoidea, Pterioidea, Anomioidea, Ostreoidea, Chamoidea, Cardioidea, Mactroidea, Tellinoidea, Solenoidea, Veneroidea, Myoidea, and Pholadoidea are very rare or entirely absent in these cave communities.

REDUCED ADULT SIZE

Cave bivalves are generally very small. Most species are less than 6 mm in adult size. This may be the result of two different processes. One is the invasion of cave habitats by species belonging to clades characterized by small body size; for example, cave bivalves belonging to the Philobryidae, Condylocardiidae, and Kelliellidae are not much smaller than closely related non-cavernicolous species in the same families. The other is true stunting, which may have occurred as a result of adaptation to such a sheltered environment. Cave species of *Huxleyia, Bentharca, Malleus (Malvufundus), Parvamussium, Cyclopecten, Chlamydella, Divarilima, Ctenoides, Limatula, Carditella, Coralliophaga,* and *Halonympha* are much smaller than all related species belonging to the same genus. In some cases (especially shallow-water genera) it is difficult to determine whether the size reduction reflects a species-level difference or is only ecophenotypic.

Pycnodonte taniguchii and *Glossocardia obesa* are exceptionally large species among the cave bivalves. They are, however, characterized by unusually reduced soft parts in comparison with the shell size.

ABUNDANCE OF DEEP-WATER ELEMENTS

One of the striking characteristics of cave bivalve communities is the dominance of deep-water genera. *Bentharca, Bathyarca, Dacrydium, Kelliella,* and *Halonympha* are important abyssal genera (Knudsen, 1970; and others), and their abundant occurrence in upper sublittoral caves is remarkable. Curiously enough, the cave species of *Dacrydium, Kelliella,* and *Halonympha* are, even though specifically distinct, morphologically very similar to northern Atlantic abyssal species (Hayami and Kase, 1993).

Huxleyia, Pronucula, Cratis, Parvamussium, Cyclopecten, Chlamydella, Divarilima, Carditella, and *Austroneaera* are also regarded as deep-water elements, because most species of these genera occur at lower sublittoral and/or bathyal depths. The discovery of many deep-water genera in sublittoral caves seems to indicate that hydrostatic pressure and water temperature are not necessarily decisive factors controlling the bathymetric distrbution of bivalve genera. The similarity in generic composition between caves and deep waters may be attributed to other environmental factors in common, such as low light levels, oligotrophic conditions, and low predation pressures.

EXPOSED LIFE OF SOME “CAVITY-DWELLING” BIVALVES

Cave bivalve communities also contain a number of species belonging to shallow-water genera, but their microhabitat and mode of life are not necessarily the same as those in non-cavernicolous environments. For example, *Barbatia decussata* (Sowerby, 1833), is one of the most ubiquitous bivalves on the rocky shores of the Palau Islands. This species predominantly dwells in narrow rock and coral cavities, whereas in the “Chandelier Cave” of the same islands, numerous individuals were found alive on the exposed wall surface. In many caves of the Ryukyu Islands, *Coralliophaga hyalina,* and *Irus* spp. are found alive on the sediment surface, notwithstanding that ordinary species of these genera are cavity-dwellers. Low predation pressure in the caves could be the most plausible explanation for the occupation of more exposed microhabitats by these species.

DOMINANCE OF PAEDOMORPHIC EVOLUTION

Cave bivalves often exhibit paedomorphic evolution. In many species of the Pteriomorphia, denticles of the provinculum are persistent through all growth stages. In the Arcidae the number of adult teeth increases with growth, but is consistently small in cave species. In many cave species of *Bentharca*, the duplivincular ligament is still undeveloped and the primary, alivincular ligament remains active throughout growth. Paedomorphosis is also apparent in *Huxleyia cavernicola* and in all the cave species of the Philobryidae, Mytilidae, and Limidae. Some authors have regarded many genera of the Philobryidae, *Dacrydium,* and *Kelliella* as neotenous, but, considering the remarkable reduction of adult size, the prevailing heterochrony in these cave bivalves should be regarded as progenesis (instead of neoteny) as defined by Gould (1977) and McKinney and McNamara (1991).

ABUNDANCE OF NON-PLANKTOTROPHIC SPECIES

The prodissoconch of cave bivalves is generally well-preserved without abrasion and erosion in both living specimens and empty shells, probably because of the low-energy conditions and oversaturation of calcium carbonate in the sea water prevailing in cave habitats. The size and shape of prodissoconch I and the presence or absence of prodissoconch II seem to indicate egg size and larval developmental mode (planktotrophic, lecithotrophic, or directly developed) to a certain extent (Ockelmann, 1965; Jablonski and Lutz, 1980, 1983).

Applying Ockelmann's (1965) criteria for the relation between prodissoconch I size and developmental type, the proportion of non-planktotrophic species among cave bivalves from the Ryukyu Islands is as high as 70% (Fig. 3). Though no reliable statistical study has been carried out for non-cryptic bivalves in this region, this value seems to be unusually high for bivalves in low-latitudinal sublittoral seas and rather comparable with the ratios of high-latitudinal (Ockelmann, 1965) and deep-water (Knudsen, 1970) faunas.

LOW FECUNDITY AND DOMINANCE OF BROODING SPECIES

Incubation of several juveniles has been well-recognized in *Cosa waikikia, Dacrydium zebra, Chlamydella incubata,* and *C. tenuissima* (see Hayami and Kase, 1993). The presence of a large, hat-shaped prodissoconch I seems to indicate that *Bentharca decorata*, most species of the Philobryidae, *Limatula kinjoi*, and *Condylocardia* sp. are also brooding species. These facts also lead us to suspect that parental care of larvae may occur in more taxonomic groups (*e.g.* Arcidae, Propeamussiidae, and Limidae) than previously realized, and that the size and shape of prodissoconch I (and also developmental strategy) are quite changeable even within one genus.

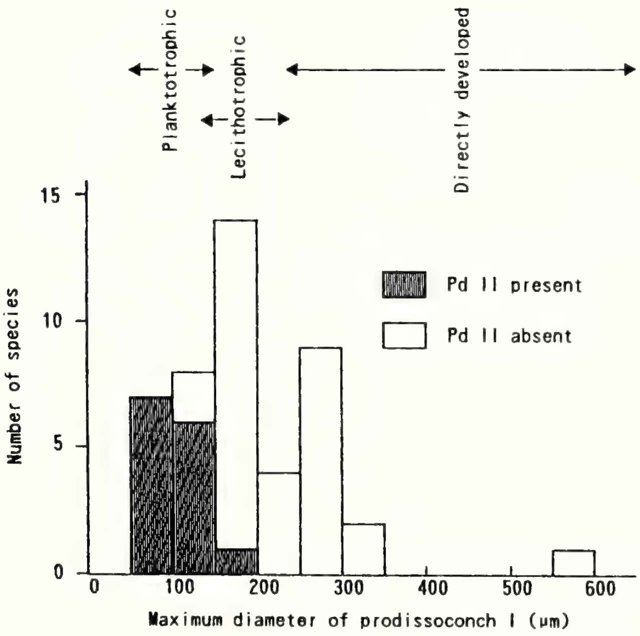

Fig. 3. Size distribution of prodissoconch (Pd) I in 45 cave bivalves from the Ryukyu Islands. Ockelmann's (1965) criteria for the types of larval development are shown above the histogram.

In addition to the dominance of brooding species, low fecundity of many cave bivalves is generally suggested by their minute adult size and large prodissoconch I. The diameter of prodissoconch I, which reflects the egg size, often exceeds 1/10 of adult size. Although little is known about their growth rate and longevity, K-strategy seems to prevail in cave bivalves. Under such nutrition-poor conditions for suspension feeders, K-selection probably becomes more advantageous than wasteful r-selection.

From a theoretical viewpoint, K-selection has been considered to be commonly accompanied by neoteny (Gould, 1977; Pianka, 1983), but this is probably not the case with the present cave bivalves, as discussed by Jablonski and Lutz (1980) for high latitudinal and deep-water mollusks. The constantly low food levels (and thus potentially low carrying capacity) could be a decisive factor controlling the adaptive strategy of cave organisms. For cave bivalves stunting and progenesis might be the only adoptable strategy to survive under food-limited conditions. Low fecundity and brooding must be also effective under such conditions. The unusually reduced soft parts of *Pycnodonte taniguchii* and *Glossocardia obesa* could also be related to the same trophic factor.

TAXONOMIC AND ECOLOGICAL PRIMITIVENESS

In addition to some "living fossil" species (*e.g. Pycnodonte taniguchii*), cave bivalves are generally archaic both in their taxonomic composition and mode of life. Although the fossil records of small-sized bivalve genera are still insufficiently known, most of the represented families are of Mesozoic or earlier origin.

The cave bivalves are mostly byssate or free-living epifaunal species, while sedentary species and deep burrowers are very rare. In the innermost part of caves *Dimyella* spp. (often accompanied by thecideidine brachiopods) are the only cementing bivalve, and all the free-living bivalves inhabit the sediment surface. Judging from core sediment samples taken in some caves of the Ryukyu Islands, the tiering of burrowing organisms is very narrow, scarcely exceeding 10 mm below the sediment surface. There are no boring gastropods (naticids and muricids), and other durophagous animals seems to be rare in these caves, although some nocturnal fish, crabs, and lobsters dwell there in the daytime.

The defenseless features of cave bivalves reminds us of the fauna before the "Mesozoic marine revolution" (Vermeij, 1977, 1987). Submarine caves are generally characterized by low predation pressure and offer suitable refuges to ecologically archaic organisms.

GEOGRAPHIC DISTRIBUTION OF MARINE CAVE BIVALVES

Among about 60 cavernicolous bivalves, several species (*e.g. Cosa uchimae, Divarilima elegans, Carditella iejimensis*, and *Halonympha asiatica*) seem to be endemic to only one or two totally dark caves of Ie Islet of the Okinawa Islands. Numerous individuals of these species have been collected there, but none was found on other islands. *Philobrya* sp. is represented by numerous living specimens from a cave of Minami-daito Island, but philobryid faunas on other islands lack this species.

Most other bivalves, including many non-planktotrophic species, however, show wide geographic distribution in the northwestern Pacific. For example, *Dacrydium zebra, Chlamydella incubata, C. tenuissima*, and *Condylocardia* sp. are certainly brooding species, but they are extensively distributed on many isolated islands around the Philippine Sea, which are far separated from one another by deep seas. *Rochefortina sandwichensis* and *Cosa waikikia*, which were originally described from Hawaii (Smith, 1885; Dall *et al.*, 1938), are known from a number of islands in this region (and also in Micronesia and the southern Pacific). Judging from the small size of prodissoconch I and the presence of prodissoconch II, the larvae of *Rochefortina sandwichensis* must be planktotrophic, but *Cosa waikikia*, at least in the Ryukyu Islands, is evidently a brooding species (Hayami and Kase, 1993: figs. 79-80).

All of the bivalves now living in these caves must have come from somewhere since the post-glacial sea-level rise, because during the Glacial Age the cave floors were above sea-level. In the Bonin Islands (Takinoura inlet of Anijima Island), some of these characteristic bivalves (including *Cosa waikikia* and *Chlamydella incubata*) were found alive in the interior of several sunken ships (dating from World War II). These facts indicate that most cave bivalves can migrate rather easily in spite of their non-planktotrophic larvae and semi-closed habitats.

It is still an unsolved problem how cave bivalves without planktotrophic larval stages were able to extend their geographic distribution. Although the diversity and distribution of such small-sized bivalves are still insufficiently studied in this region, we hypothesize the following three possibilities for the wide geographic distribution of cave bivalves.

1. It is possible that these cave bivalves are extensively distributed and not necessarily indigenous to such a cryptic environment.
2. Developmental mode of bivalves could be more plastic than presently recognized. Ancestral planktotrophic populations could change their developmental strategy rapidly as an adaptation to caves.
3. Minute epibyssate species, even adult individuals, could be able to migrate for a long distance by rafting. They could attach themselves to small floating objects or swimming animals.

At present, possibilities 1 and 2 cannot necessarily be denied, but, we suppose, they are rather unlikely for the following reasons. Empty shells of a few cavernicolous bivalves are occasionally found in beach sands of this region, but the species assemblages of minute bivalve shells in sediments on the beach and open sublittoral bottom are almost entirely different from those in the present cave samples. So far as we have examined, the size and shape of the prodissoconch (as well as adult morphology) in each cave species are considerably stable througout the samples from distant islands. Parallel change of developmental mode at the adaptation to each cavernicolous habitat is, therefore, highly unlikely.

Ó Foighil (1989) extensively examined the developmental mode and geographic distribution of the cosmopolitan intertidal byssate bivalve genus *Lasaea* based on more than 150 museum samples. The results indicated that brooding species of *Lasaea* releasing crawl-away juveniles show wider geographic distribution than planktotrophic species of the same genus. It was pointed out that pelagic larvae are not necessary for long-distance dispersal in that genus, and that rafting has played a key role for the dispersal mechanism.

Rafting, as considered by several authors (Soot-Ryen, 1960; and others), could be an alternate dispersal method especially for minute byssate bivalves. Most of widely distributed cave bivalves without planktotrophic stages are small-sized and epibyssate like *Lasaea* species. In Miyako and some other islands we have actually observed that a large number of living individuals of *Cosa waikikia* and *Chlamydella incubata* attach themselves to the exposed part of annelid tubes (Hayami and Kase, 1993: fig. 212) and soft sponges. Although there is no direct evidence, rafting could be the most important method for long-distance dispersal of these cave bivalves.

Iliffe and his coworkers surveyed marine troglobites (mainly crustaceans) in the subtropical northern Atlantic (Caribbean Sea, Bermuda, and Canary Islands), elucidating that several relict species inhabit the caves of these distant islands in common (Iliffe *et al.*, 1983, 1984). Ocean-floor spreading was once hypothesized as a cause for the amphi-Atlantic distribution of several relict crustaceans (Hart *et al.*, 1985). Furthermore, Iliffe (1990) discussed the possibility that the transoceanic dispersal of marine cave faunas could occur by way of deep-water caves and crevicular habitats. The hypothesis seems to be supported by the taxonomic resemblance between cave and deep-water faunas.

As noted before, a number of bathyal and abyssal genera are represented in the present cave bivalves. This is indeed one of the most remarkable characteristics, suggesting that the cave bivalves are, at least in part, of deep-water origin. None of them, however, is strictly identical with any described deep-water species. Although we can by no means deny the possibility of gene flow between distant sublittoral caves via deep-sea bottom, there is not much evidence to support this dispersal method.

CONCLUDING REMARKS

The bivalves collected from a number of marine sublittoral caves in several isolated islands around the Philippine Sea show striking and common characteristics such as reduced adult size, paedomorphosis by progenesis, dominance of non-planktotrophic larval development, and low fecundity. These features are probably related to one another and could have resulted from a common adaptive strategy to cavernicolous environments. Low food levels and low predation pressure are certainly the most important controlling factors. This conclusion seems to be also supported by the taxonomic resemblance of this fauna to deep-water bivalves and by the ecological archaism similar to the faunas before the "Mesozoic marine revolution."

Furthermore, the characteristics of cave bivalves seem to suggest the type of adaptive strategy that could be advantageous when extremely oligotrophic conditions prevail in the world. Although many problems (especially the process of migration and speciation) remain unsolved, we anticipate that the marine cave biota offers substantial data to test various theories in evolutionary biology.

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LITERATURE CITED

Dall, W. H., P. Bartsch, and H. A. Rehder. 1938. A manual of the Recent and fossil marine pelecypod mollusks of the Hawaiian Islands. *Bulletin of the Bernice P. Bishop Museum* 153:1-233.

Gould, S. J. 1977. *Ontogeny and Phylogeny*. Harvard University. Cambridge, Massachusetts. 501 pp.

Hart, C. W. Jr., R. B. Manning, and T. M. Iliffe. 1985. The fauna of Atlantic marine caves: evidence of dispersal by sea floor spreading while maintaining ties to deep waters. *Proceedings of the Biological Society of Washington* 98:288-292.

Hayami, I. and T. Kase. 1992. A new cryptic species of *Pycnodonte* from Ryukyu Islands: a living fossil oyster. *Transactions and Proceedings of the Palaeontological Society of Japan* (n. s.) 165:1070-1089.

Hayami, I. and T. Kase. 1993. Submarine cave Bivalvia from the Ryukyu Islands: systematics and evolutionary significance. *University Museum, University of Tokyo, Bulletin* 35:1-133.

Iliffe, T. M. 1990. Crevicular dispersal of marine cave faunas. *Mémoires de Biospéologie* 17:93-96.

Iliffe, T. M., C. W. Hart, Jr., and R. B. Manning. 1983. Biogeography and the caves of Bermuda. *Nature* 302:141-142.

Iliffe, T. M., H. Wilkens, J. Parzefall, and D. Williams. 1984. Marine lava cave fauna: composition, biogeography and origins. *Science* 225:309-311.

Jablonski, D. and R. A. Lutz. 1980. Molluscan larval shell morphology: ecological and paleontological applications. *In: Skeletal Growth of Aquatic Organisms*, D. C. Rhoads and R. A. Lutz, eds. pp. 323-377. Plenum Press, New York.

Jablonski, D. and R. A. Lutz. 1983. Larval ecology of marine benthic invertebrates: paleobiologic implications. *Biological Review* 58:21-89.

Jackson, J. B. C., T. F. Goreau, and W. D. Hartman. 1971. Recent brachiopod-coralline sponge communities and their paleoecological significance. *Science* 173:623-625.

Kase, T. and I. Hayami. 1992. Unique submarine cave mollusc fauna: composition, origin and adaptation. *Journal of Molluscan Studies* 58:446-449.

Knudsen, J. 1970. The systematics and biology of abyssal and hadal Bivalvia. *Galathea Report* 11:7-241.

McKinney, M. L. and K. J. McNamara. 1991. *Heterochrony: The Evolution of Ontogeny*. Plenum Press, New York. 437 pp.

Moore, D. R. 1970. A new genus and species of Dimyidae from the Caribbean coast of Mexico. *Journal de Conchyliologie* 107:137-142.

Ockelmann, W. K. 1965. Developmental types in marine bivalves and their distribution along the Atlantic coast of Europe. *Proceedings of the First European Malacological Congress, London, 1962*, L. R. Cox and J. F. Peake, eds. pp. 25-35. Conchological Society of Great Britain and Ireland and Malacological Society of London, London.

Ó Foighil, D. 1989. Planktotrpohic larval development is associated with a restricted geographic range in *Lasaea*, a genus of brooding, hermaphroditic bivalves. *Marine Biology* 103:349-358.

Pianka, E. R. 1983. *Evolutionary Ecology, 3rd ed.* Harper and Row, New York. 337 pp.

Smith, E. A. 1885. Report on the Lamellibranchia collected by *H. M. S. Challenger* during the years 1873-76. *Report on the Scientific Results of the Voyage of H. M. S. Challenger 1868-1876, Zoology* 13:1-341.

Soot-Ryen, T. 1960. Pelecypods from Tristan da Cunha. *Results of Norwegian Scientific Expedition, Tristan da Cunha* 5:1-47.

Vermeij, G. J. 1977. The Mesozoic marine revolution: evidence from snails, predators and grazers. *Paleobiology* 3:245-258.

Vermeij, G. J. 1987. *Evolution and Escalation: an Ecological History of Life*. Princeton University Press, Princeton, New Jersey. 527 pp.

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Retention around and long-distance dispersal between oceanic islands by planktonic larvae of benthic gastropod Mollusca

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Abstract: Indo-Pacific species of gastropod mollusks often have very wide geographic distributions sometimes extending halfway around the world, from the Marquesas or Hawaiian Islands westward throughout the tropical Pacific and Indian Oceans to the Red Sea and eastern African coast. For a gastropod species to attain such a wide distribution and yet also maintain genetic continuity among so large a number of disjunct populations, some sort of dispersal must occur among the many scattered oceanic islands within the species range. One way this can be accomplished is by long-distance dispersal of planktonic larvae. It is proposed that a small fraction of the total veligers produced by a gastropod species on any particular oceanic island, such as the island of Hawaii, will escape the effect of local circulation and as a consequence will have the possibility to be passively transported by oceanic currents to other remote islands. Evidence from plankton tows taken in proximity to Hawaii, when related to a knowledge of local mesoscale circulation, shows how larvae can be dispersed outside the influence of local island circulation. At the same time, plankton samples from the tropical central and western Pacific Ocean reveal how gastropod veligers are passively advected over long distances by ocean currents. Furthermore, drift bottle data illustrate how gastropod veligers, once entrained within the North Equatorial Current, can encounter other oceanic islands and give evidence for the probability of such an island encounter.

Notwithstanding evidence that larvae can be dispersed away from their native island (in this instance from the island of Hawaii), it is inferred that gastropod species are largely self-sustained by veligers from indigenous populations. For such recruitment from local populations to occur, veligers must (1) be constrained by the local hydrography to remain within the proximity of their natal island, (2) survive the vicissitudes of planktonic life in sufficient numbers and complete development to the competent stage when settlement and metamorphosis become possible, and (3) must be returned passively to a suitable sublittoral environment by the local circulation. Evidence from plankton tows, a knowledge of mesoscale circulation, and data from drift-bottle returns allow an explanation of how larvae can be retained and how they are ultimately returned to their island of origin. Paradoxically, it seems that the hydrological phenomena that sometimes return larvae to their natal island can, in other instances, passively transport veliger larvae out to sea.

Oceanic islands, because of their spatial isolation and as a consequence of their volcanic origin from the floor of the ocean, present unusual opportunities for the study of dispersal and its biogeographic and evolutionary consequences (Clague and Dalrymple, 1989; Walker, 1990). A number of questions immediately arise. Are marine invertebrates on isolated oceanic islands mostly endemic species or are such islands inhabited predominantly by very widely distributed species? How are oceanic islands initially populated? How do island populations once established maintain themselves in apparent isolation? Can island populations maintain themselves principally by the recruitment of their own larvae?

An insight into the distributional patterns found among marine gastropod mollusks on oceanic islands can be gained by considering the family Architectonicidae. Bieler (1993) has shown that the geographic range of *Psilaxis radiatus* (Röding, 1798) encompasses oceanic islands throughout the tropical Pacific from the Marquesas and Hawaiian Islands westward to the Indian Ocean and eastern Africa and northwestward into the Red Sea (Fig. 1). Among the 20 species of Architectonicidae known to occur on the Hawaiian Islands, three-fourths have geographic ranges very similar to that of *P. radiatus*, extending almost halfway around the world. Such very wide geographic distributions among contemporary Indo-Pacific species can only be explained by some form of long-distance dispersal (Scheltema 1989, 1992).

The Architectonicidae are not an isolated example. The Cypraeidae (cowries) also are widely distributed throughout the Indo-Pacific. Among the 16 Holocene Indo-Pacific genera enumerated by Kay (1995: 218; table 1), 15 are known to include species with planktonic larvae. Schilder (1969) listed 11 species from Hawaii among which eight are known also from eastern Africa. Indeed, some Cypraeidae that occur in the Indian Ocean and tropi-

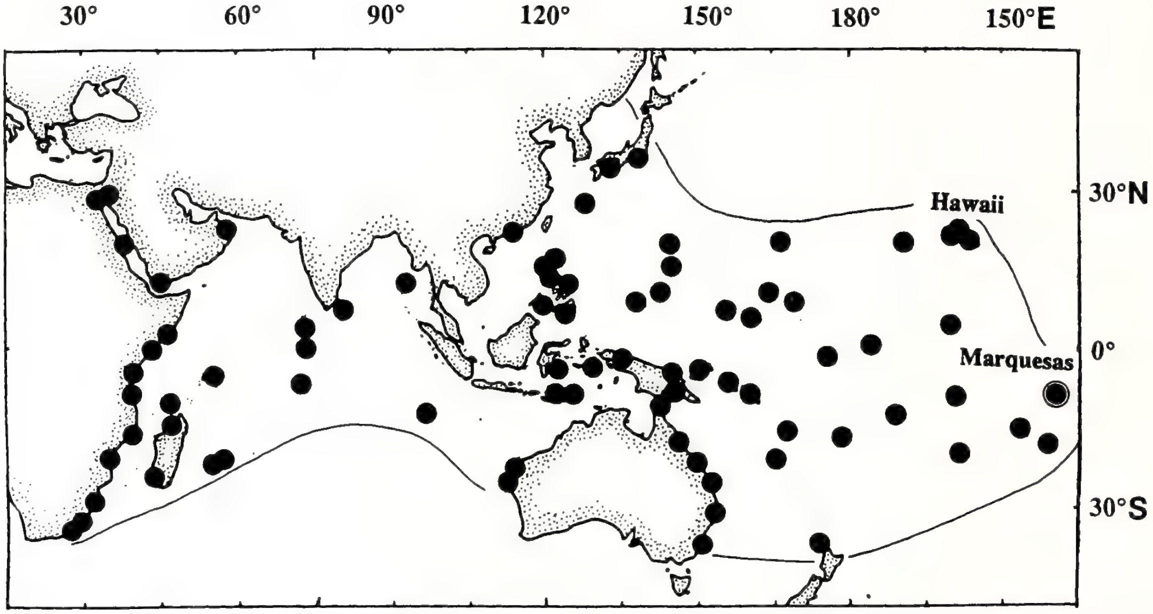

Fig. 1. Geographic distribution of *Psilaxis radiatus* (Architectonicidae). The species range extends from the Marquesas (encircled point) to eastern Africa and the Red Sea. Two additional locations in the eastern Pacific not shown are considered by Bieler to be from non-reproducing, expatriot populations. The continuous line delimits the approximate range of the species. (Modified after Bieler, 1993).

cal western and central Pacific are known also from the eastern Pacific having successfully breached the "eastern Pacific barrier." Emerson and Chaney (1995) have shown that among the 24 eastern Pacific cypraeid species representing eight genera, 15 also are known from the Indo-Pacific. Eight among these 15 amphi-Pacific species are found not only upon eastern Pacific islands (*i. e.* Clipperton, Revillagigedo, Cocos, and Galápagos) but also on the continental shores of central and tropical South America. Some other gastropod families also known to include species with similarly wide geographic distributions throughout the Indo-Pacific region are the Ranellidae (*e. g. Cymatium nicobaricum* Röding, 1798), the Coralliophilidae [*e. g. Quoyula mondata* (Blainville, 1882)], and the Naticidae (*e. g. Natica marochiensis* Gmelin, 1781). Examples given here are but a few specific instances of gastropod species that have large geographic ranges and occur commonly on widely scattered tropical islands.

If indeed oceanic islands are inhabited mostly by broadly distributed species, this fact should be reflected by a low percentage of species endemism. A few examples can be considered here. In waters of the Hawaiian Islands, among the most isolated of central Pacific archipelagos, only 19% of all marine gastropods are endemic. Moreover, among the non-endemic species most are Indo-Pacific (66% of the total) while the remainder (15% of total) are Pacific "Plate" species. Paulay (1989) encountered on remote Pitcairn Island (25°S, 130°W) an attenuated Indo-West-Pacific marine gastropod fauna with only 2% endemism. Likewise, Kay (1971) found on Line Island (directly in the path of the North Equatorial Current) an endemism of about 2%. Such low endemism is in sharp contrast with land snails which lack an effective mode of regular dispersal and among which endemism can exceed 90% (Kay and Palumbi, 1987).

The extraordinarily large geographic range illustrated by the examples offered above along with the correspondingly low endemism encountered on oceanic islands argues for the hypothesis that long-distance dispersal must be commonplace among tropical sublittoral gastropods and that the initial colonization as well as genetic continuity among marine gastropods must be maintained largely by long-distance dispersal, presumably by the dispersal of teleplanic larvae.

Contemplate now the other side of the problem. How are populations of oceanic islands maintained? Are they self-sufficient and, if so, how is this accomplished? The answer necessarily must take into account the role of passively dispersed larvae, but in this instance instead of being transported away, there must be instead a way to retain larvae over the period of their development and subsequently to return them to their natal island. But how can some larvae be carried away while at the same time others are retained and returned to their native island? It is the purpose of this paper to explain this seeming paradox.

METHODS

The distribution of teleplanic gastropod veligers in open waters of the tropical Pacific Ocean was determined from examination of 328 oblique plankton tows among which 174 were from collections of the Scripps Institution of Oceanography (SIO) obtained during various expeditions over a period of 26 years (see Scheltema, 1986: 243 for a detailed list of expeditions and stations). The remaining 154 samples were 20-min oblique tows to ca. 150 m taken at 1.5-2.5 knots with a 0.75-m net having ca. 0.3-mm mesh. Included were samples from the following expeditions: KNORR, voyage 73 (1979), New Zealand to Hawaii, 36 samples; the Papatua Expedition (1985), Manzanillo, Mexico, to American Samoa, 33 samples; the Helios Expedition (1987), San Diego, Gambier Islands, Pitcairn Island, Austral Islands, and Tahiti (Society Islands), 50 samples; Hydros Expedition (1989), tropical waters between San Diego and Juan Fernández Islands, 35 samples. The samples were reduced to a volume of 0.5-1.0 l, depending on the density of organisms, in 5% formalin with sodium borate added. On the Helios and Hydros Expeditions, all invertebrate larvae were sorted to family at sea. Gastropod veligers were separated and preserved in 80% alcohol with sodium borate added to control pH.

The locations of sampling stations along the western coast of the island of Hawaii were determined by the hydrography at the time of the study. Plankton tows were taken at night (2240-0530 hrs) between July 23 and 26, 1982, at 1-1.5 knots. The samples were inspected in the laboratory and all veliger larvae removed. Current velocity and direction were determined by radio-tracked Lagrangian drifters. Vertical temperature profiles were determined by expendable bathythermographs (XBT) (see Lobel and Robinson, 1986, 1988, for further details).

RESULTS AND DISCUSSION

Long-distance dispersal

Can long-distance transport of planktonic larvae explain the observed geographic distribution of gastropod species among oceanic islands? What evidence is needed to infer that long-distance larval dispersal occurs? First, it must be shown that planktonic larvae are actually found far out at sea drifting passively with ocean currents. Two examples will suffice to show that larvae indeed do occur in the major ocean currents. Inasmuch as most members of the family Architectonicidae already have been shown to be widely distributed geographically, it is not too surprising to learn that veligers belonging to species of this family are

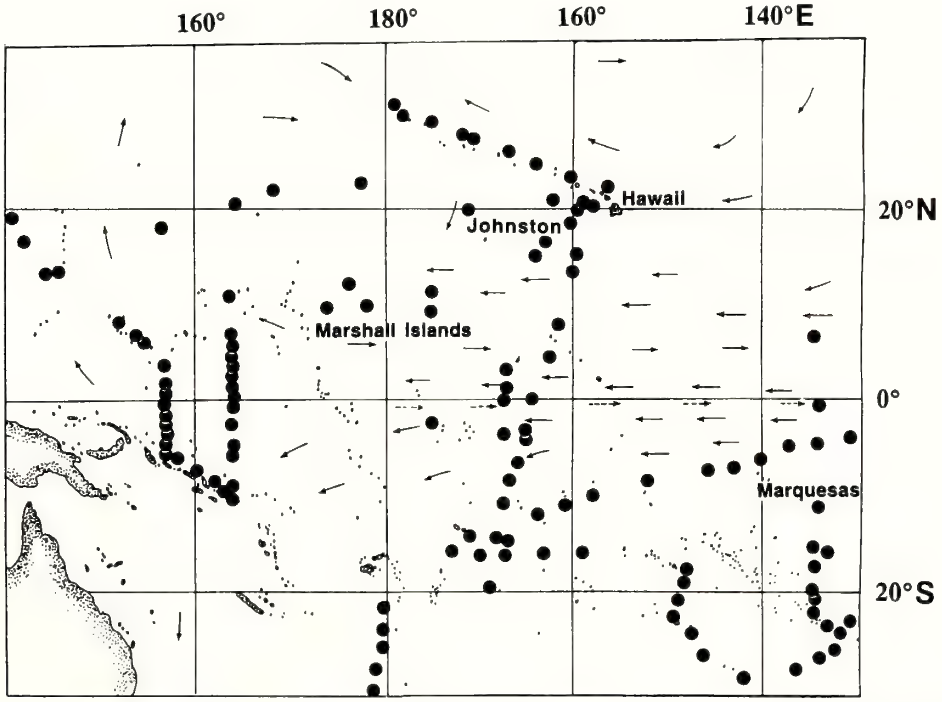

Fig. 2. Distribution of veliger larvae belonging to the gastropod family Architectonicidae from plankton samples taken between the surface and *ca.* 150 m in the tropical Pacific Ocean using a 3/4 - m net with 0.3 mm mesh (see Scheltema and Williams, 1983).

frequently encountered in the tropical Pacific Ocean (Fig. 2). Likewise, larvae of cypraeid species also have been found distributed over large areas of the central Pacific (Fig. 3).

Second, to approximate the distance that larvae can be advected by an ocean current it is necessary to know the minimum time required to attain competence to metamorphose. There is, however, a paucity of observations on the life histories of tropical Pacific gastropods owing to the difficulty encountered in rearing them in the laboratory. Although teleplanic larvae have a long development as can be inferred from their position of capture in the plankton (Scheltema, 1971, 1992, 1995), the maximum length they are able to delay metamorphosis is usually not known. Govan (1994: 55) estimated that among several species of Pacific *Cymatium*, three months is required to reach competence to settle, but to this must be added the potential for delay of metamorphosis in the absence of a cue for settlement.

Mitton *et al.* (1989) questioned whether gastropod veligers can retain their ability to metamorphose for long periods of time after attaining competence. Evidence from laboratory experiments has shown that certain prosobranch gastropod species can delay settlement long after attaining the competence to metamorphose and that in the absence of a suitable cue can delay settlement and increase two- to three-fold the length of their planktonic life (Scheltema, 1956, 1961). The opisthobranch *Aplysia juliana* Quoy and Gaimard, 1832, has been shown in the laboratory to delay settlement and metamorphosis up to 300 days (Kempf, 1981). Evidence from larvae taken far out at sea and held in the laboratory confirm that larvae can retain their ability to metamorphose (Scheltema, 1986: 242). Combined evidence from field and laboratory observations provide estimates of the possible delay in settlement of ten species of teleplanic gastropod veligers that range between 30 and 138 days giving a total pelagic life of 55-320 days (Scheltema, 1971).

Third, it is necessary to know the direction and velocity of the major ocean currents likely to passively disperse gastropod larvae. The general circulation of the tropical Pacific is now fairly well known (*e. g.* Tomczak and Godfrey, 1994). Flowing from east to west are the north and south equatorial currents. Between these two westerly moving currents is a seasonally occurring west to east equatorial countercurrent prominent during the summer but much reduced or entirely absent during some winter months (Wyrtki, 1966; Toole *et al.*, 1988). There is in addition an equatorial undercurrent flowing toward the east between 50 and 100 m depth at a temperature between 20° and 25°C, sufficient for survival of tropical veliger larvae (Knauss, 1970). The velocities of the major equatorial Pacific surface currents generally fall between 25 and 90 cm/sec but can be

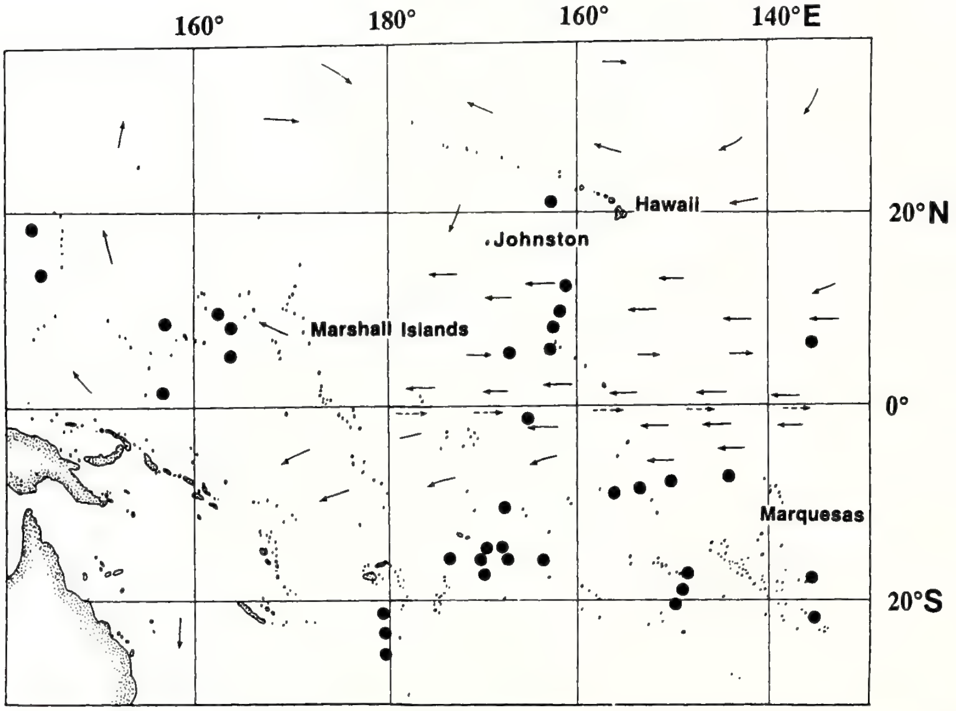

Fig. 3. Distribution of veliger larvae of the gastropod family Cypraeidae from plankton samples taken between the surface and *ca.* 150 m in the tropical Pacific Ocean (samples as in Fig. 2).

as high as 125 cm/sec in the equatorial undercurrent. Advection by ocean currents provides quasi-permanent or seasonally reoccurring corridors for the passive transport of planktonic veliger larvae and will largely limit the direction and velocity of larval dispersal. Smaller scale details can add some complexity (McNally *et al.*, 1983) but will not substantially alter the major direction of dispersal. Larvae can be readily transported eastward or westward but not northward or southward across the equatorial region except periodically during the time of El Niño (see Richmond, 1990).

If one assumes minimum and maximum current velocities between 25 cm/sec and 125 cm/sec, then in three months [the time required for a *Cymatium* species to attain competence to settle (Govan, 1994)], the median distance a larva can be transported is about 6000 km. This value assumes a straight trajectory, which is unlikely, but gives at least an estimate of the possible magnitude of larval dispersal, even when no delay in metamorphosis is assumed. Under favorable conditions larvae readily could be dispersed for much greater distances.

But what is the probability that a larva will actually encounter another island? Obviously, because it is not possible to follow an individual larva for thousands of kilometers, no direct answer is possible but reasonable inferences can be made from drift-bottle data. The release of 980 bottles made directly within the north and south equatorial currents between 175° and 192° E longitude resulted in a 2.9% recovery. All returns were from islands in the western Pacific including the Solomons, New Guinea, the Carolines, and Gilberts (Scheltema, 1986, from data of Barkley *et al.*, 1964). When bottles released in the central Pacific outside the major east-west currents were included (a total of 2127 bottles) then the percentage of recovery dropped to 1.3%.

Drift-bottle data can provide a "drift coefficient," *i. e.* the likelihood that a passively drifting larva in the open sea will ultimately encounter another island. The probability that the offspring from any particular female will actually be dispersed to another island will depend also upon (1) the fecundity of the individual which will vary both with the species and size of the individual, and (2) the survival of the larvae during dispersal.

Published estimates of fecundity among tropical gastropods are rare. It has been estimated that an egg mass of the architectonicid *Heliacus variegatus* (Gmelin, 1791) contains 30,000 embryos (Bieler, 1993), but it is not known how many egg masses are produced by one female. Some species of Cypraeidae can produce up to 500,000 veligers from a single egg mass (Emerson and Chaney, 1995: 15). Estimates of fecundity of several species of *Cymatium* range from a minimum of 100,000 to over one million eggs per female (Govan, 1994).

Consider now an individual female with one-half million gametes. Even if one uses conservative values, *e. g.* a drift coefficient of 0.013 and assumes a survival of 0.1%, there is a likelihood that at least some larvae (ca. 6) will reach another island (see Scheltema, 1978, 1986, for further details). However, if in its lifetime an individual survives to reproduce more than once, there is the possibility that more than one-half million eggs will be produced. Moreover, if the entire population of an island is considered, the chances that some larvae will be dispersed and encounter another island will increase accordingly.

Larval distribution from plankton samples and calculations based on drift coefficients and known current velocities allow the conclusion that islands within the proximity of major ocean currents will regularly encounter a small but more-or-less continuous flux of larvae, probably sufficient to maintain genetic continuity between islands (see Lewontin, 1974: 213). It is not, however, nearly so evident how larvae could reach some of the more remote oceanic islands not directly in a major ocean current.

Retention of larvae around islands

Consider now the converse question, viz. how are populations of gastropods sustained on oceanic islands? To address this problem once again it is necessary to consider surface currents and their effect upon the spatial disposition of planktonic larvae. The island of Hawaii will serve as an example.

Sea surface to the lee of the island of Hawaii is dominated by eddies varying considerably in size, number, and location (Patzert, 1969). Such eddies can be nearly circular or elliptical, and range between 50 and 150 km in diameter. They are predominantly cyclonic, *i. e.* rotating in a counterclockwise direction, driven by the prevailing northeasterly and easterly winds that blow through the restricted passage between the islands of Maui and Hawaii. Although there are some seasonal differences in wind direction and velocity (Blumenstock and Price, 1994: 101, table 4), being more persistently from the north-northeast to east and at greater velocity during the summer months, nevertheless, eddies have been observed in all seasons of the year (Patzert, 1969: table 1).

The eddies can be located either by drogues, inferred from temperature distribution, or derived from the dynamic topography determined by standard oceanographic methods (Fig. 4). The formation of an eddy could require from "approximately two weeks for a weak eddy to a month for a more intense eddy" (Patzert, 1969: 45) and during its lifetime of three or more months it may move up to 350 km in a westerly or northwesterly direction at an average rate of 5.2 km/day, increasing in size as it moves out to sea where finally it is completely dissipated. This latter process is still poorly understood and was discussed in more detail by Patzert (1969).

The rotation rate of the eddy is related inversely to the depth of the 20°C isotherm and hence the pressure gradient which determines the current velocity and hence the rate of rotation. When the 20°C isotherm is at 70 m depth, the rate of rotation is approximately 3.5 to 4.5 days; when the 20°C isotherm is at about half this depth (30-40 m), the rate of rotation is approximately doubled, to 6 or 7 days (see Patzert, 1969: 14, table 4).

How will such eddies affect the distribution of larvae and their retention around islands? One outcome can be that veligers become entrained and thereby gain time to complete their development to the competent stage when settlement and metamorphosis becomes possible. But does this actually happen? Are gastropod larvae actually entrained in mesoscale eddies? Some initial evidence comes from a series of plankton tows taken between July 23 and 26, 1982, in an eddy off Keahole Point, Hawaii (Fig. 5).

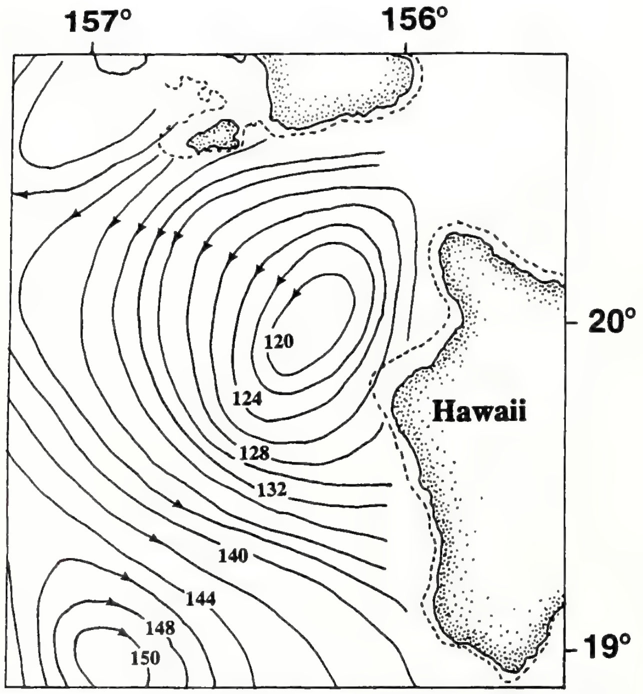

Fig. 4. Dynamic topography (0/500 db) off the Kona Coast of the island of Hawaii in May 1965, to illustrate a large cyclonic eddy. Note also smaller anticyclonic eddies (modified after Patzert, 1969: fig. 12). The eastern edge of the large cyclonic eddy intersects the shoreline on the Kona Coast and could carry gastropod veligers back to their parent populations.

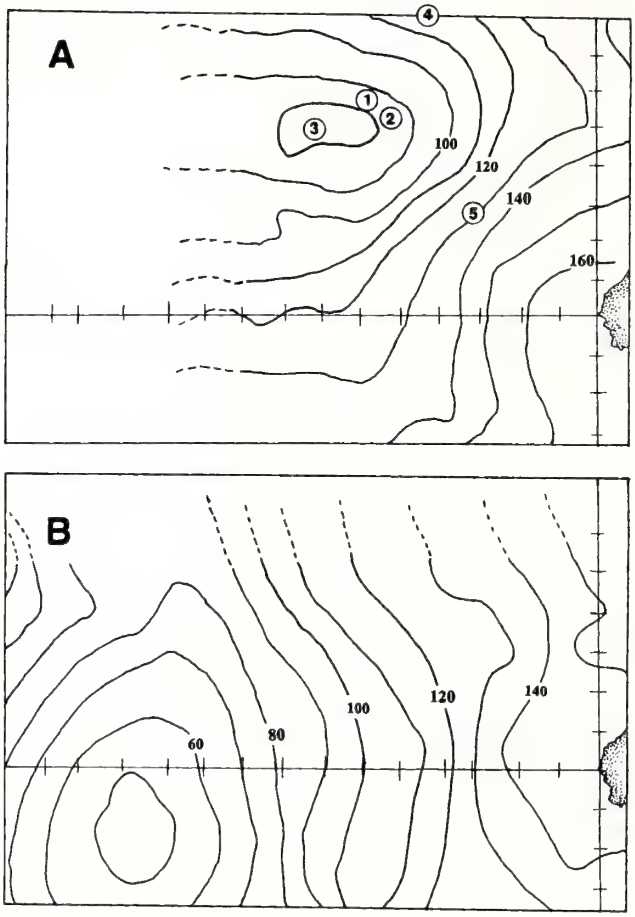

Fig. 5. Cyclonic eddy, Keahole Point, off the western shore of Hawaii during July as defined by the depth of the 20°C isotherm. Contour intervals are 10 m; intervals along scale are 7 km. **A.** Eddy during July. Plankton sampling locations are indicated by circled numerals. **B.** Same eddy in September (modified after Lobel and Robinson, 1986: fig. 3). Locations 1, 2, and 3 are at the center of the eddy (see Table 1); locations 4 and 5 are at the outer edge. Samples were taken at night using a 1-m net with 0.5-mm mesh (see Lobel and Robinson, 1986: 492 for details).

Samples from five different locations within an eddy, including three from its center and two near its edge and taken from the depth of the 24°C isotherm, contained a variety of invertebrate larvae, among them veligers representing 12 gastropod families (Table 1) and including teleplanic species found in the open ocean, *e. g. Psilaxis radiatus* (Architectonicidae); *Cypraea isabella* Linné, 1758 (Cypraeidae); *Natica gualteriana* Recluz, 1844 (Naticidae); *Carinapex minutissima* (Garrett, 1873) (Turridae); and *Strombus maculatus* Sowerby, 1842 (Strombidae). Larvae are reported here (Table 1) only to family because at present many individuals cannot be reliably identified to species (but see Taylor, 1975). Nonetheless, most specimens are readily recognized to belong to taxa commonly found among the teleplanos.

What will happen to the larvae entrained in an eddy? How will their residence within the eddy affect their retention and possible recruitment to the island of the parent population? Two possible outcomes are proposed. One

Table 1. Families of Gastropoda represented in samples from a mesoscale eddy off the island of Hawaii, July 23-26, 1982 (station numbers refer to positions indicated on chart, Fig. 5)*.

	Eddy Center			Edge of Eddy	
	1	2	3	4	5
Architectonicidae	X	X	X	X	X
Columbellidae		X	X	X	X
Conidae				X	
Coralliophilidae	X	X	X	X	X
Cypraeidae	X	X	X	X	X
Lamellariidae	X	X	X	X	X
Naticidae	X	X	X	X	X
Ranellidae	X	X	X	X	X
Thaididae	X	X	X	X	X
Strombidae	X	X	X	X	X
Triphoridae				X	X
Turridae	X	X	X	X	X
Unidentified	X	X	X	X	X

* Station 1 - July 23, 20° 00.5' N, 156° 25' W
Station 2 - July 24, 20° 09.5' N, 156° 19.5' W
Station 3 - July 24, 20° 00' N, 156° 23.5' W
Station 4 - July 25, 19° 51.5' N, 156° 15' W
Station 5 - July 26, 19° 51.5' N, 156° 15.5' W

alternative is that veliger larvae are returned to their natal island when the periphery of an eddy intersects the region between 200 m and the shoreline (Lobel, 1989). The anticyclonic eddy along the Kona Coast shown in Fig. 4 illustrates how the usually intermittent and oscillatory tidal current that occurs along the coast is replaced by a strong northerly along-shore current (*i. e.* the eastern edge of the eddy) that could return larvae back to their parent population. Indeed, such currents have been demonstrated empirically by moored current meters (*e. g.* see Robinson and Lobel, 1985).

Return of larvae to the Kona Coast will be determined by (1) the rotation rate of an eddy which has been observed to vary between 4 and 8 days (Patzert, 1969), and (2) the time required for a larva to complete development and gain competence to settle. Consequently, so long as the eddy remains more-or-less stationary and its edge intersects the Kona coastline (Fig. 4) every fourth day of rotation a larva will have the possibility to settle in response to a cue from the bottom. Consequently, larvae with short development times will have ample opportunity to settle along the coast. On the other hand larvae with long development times, for example species of *Cymatium* with a development of three months, could be retained near the island going through numerous rotations of the eddy before they can settle and metamorphose. Rotation of the mesoscale eddy is such that under favorable circumstances both rapidly and slowly developing larvae can be retained and returned to the coastal environment.

A second alternative is that larvae, instead of returning to their natal island, are carried along as the eddy moves out to sea. This seems to have happened to the larvae in the eddy sampled by us off the Kona Coast (Fig. 5). On the upper chart (A) the position of the eddy is shown at the end of July when the plankton tows were taken; the lower chart (B) shows the same eddy in September; it had enlarged between July and September and was moving westward. It may be inferred that most of the entrained larvae were carried out to sea and with the ultimate decay of the eddy these veligers were released to be dispersed subsequently in a generally westward direction by surface currents. The destinies of such ocean-bound veligers are determined by the velocity and direction of the ocean surface currents into which they are "released" and upon their ability to delay metamorphosis until by chance they should encounter some distant island.

Yet, other evidence in support of the hypothesis that larvae can be retained and returned to their natal population comes, as with long-distance dispersal, from the release and recovery of drift bottles. Barkley *et al.* (1964) released more than 4000 drift bottles during 1962, mostly in the region bounded by 18° to 23° N and 154° to 161° W, that is, the area surrounding the major islands of the Hawaiian chain. Recoveries from this experiment show bottles released in proximity to an island will tend to be recovered subsequently on the shore of nearby islands (Table 2; Fig. 6). There were seasonal differences in the percent recovery, the highest (12.5%) occurring between March and May. At other times of the year many fewer bottles were returned, between 2.7 and 6.0%. Even so, the mean annual recovery, 5.5%, was remarkably high for this kind of study. If it is assumed that drift-bottle returns reflect the passive dispersal of veligers, then a simple calculation can be made as before to show the likelihood that some larvae from each individual female will be returned to their parent population. A species such as *Cymatium nicobaricum* (Röding, 1798), estimated to spawn more than 500,000 eggs per female (Govan, 1994: 54), even with only 0.1% survival

Table 2. Drift bottles released in a quadrangle bounded by 18° to 23° N and 154° to 161° W in 1962 and subsequently recovered on islands of the Hawaiian Archipelago (data from Barclay *et al.*, 1964).

Time of year	No. of release points*	Total no. bottles released	Percent of bottles recovered
Jan. - Feb.	180	1005	2.7
March - May	59	925	12.5
June - July	182	1840	3.5
Sept. - Dec.	–	200	6.0

* From March through May returns were received from 52.5% of release points. During other parts of the year returns were received from only 9-11% of release points.

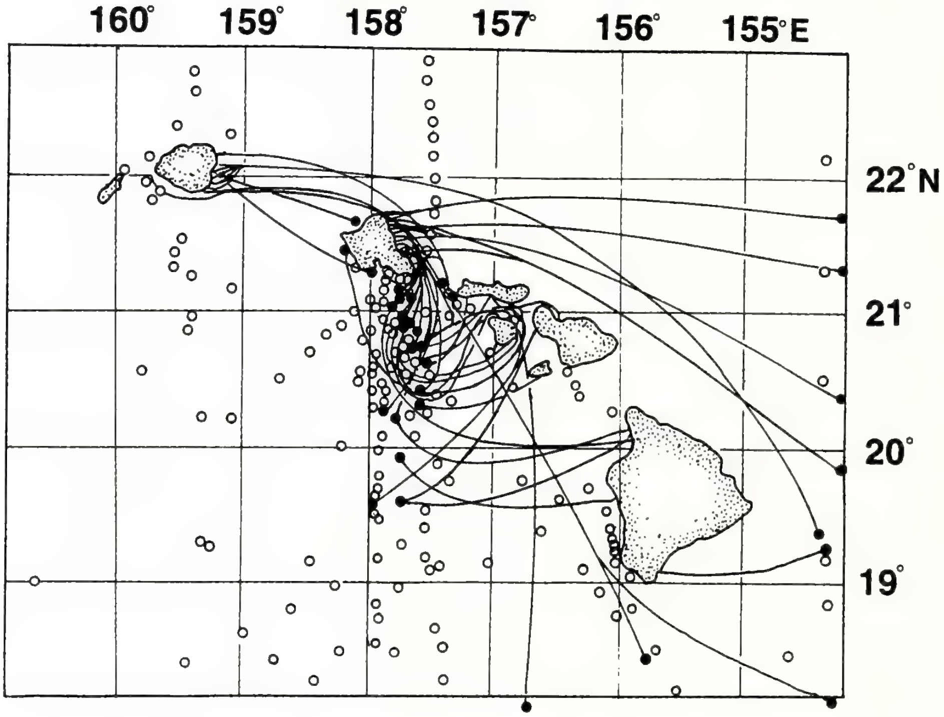

Fig. 6. Drift bottles released and recovered between January and June, 1962 (the time of highest returns), in a region bounded by 18° and 23° N and 154° and 161° W (modified after Barkley *et al.*, 1964: fig. 4-7). See also Table 2. Closed circles (●) indicate points of release from which returns were received; lines show probable route of drift bottles from point of release to location of recovery; open circles (O) show points of release from which no returns were received.

can reasonably be expected to have surviving offspring if 5.5% are returned to their parent island. On average most species found on oceanic islands by such calculations will be able to sustain an indigenous population.

CONCLUSIONS

There is much speculation in the foregoing account; obviously there remains much to be done, *e. g.* genetic studies using molecular techniques could support or refute some of the conclusions made here. One, nevertheless, must marvel at the remarkable means by which some larvae are apparently retained to sustain their parent population while others evidently are transported out to sea to colonize or to provide genetic continuity with other remote islands, and that both the dispersal and retention of larvae can be governed by the rotation and movement of mesoscale eddies.

ACKNOWLEDGMENTS

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LITERATURE CITED

Barkley, R. A., B. M. Ito, and R. P. Brown. 1964. Releases and recoveries of drift bottles and cards in the Central Pacific. *U. S. Fish and Wildlife Service Special Scientific Report* 492: 31 pp.

Bieler, R. 1993. Architectonicidae of the Indo-Pacific (Mollusca, Gastropoda). *Abhandlungen der Naturwissenschaftlichen Verein Hamburg (NF)* 30: 337 pp.

Blumenstock, D. L. and S. Price. 1994. Climates of the states: Hawaii. *In: A Natural History of the Hawaiian Islands, Selected Readings II,* E. A. Kay, ed. pp. 94-114. University of Hawaii Press, Honolulu.

Clague, D. A. and G. B. Dalrymple. 1989. Tectonics, geochronology, and the origin of the Hawaiian - Emperor Volcanic Chain. *In: The Geology of North America: The Eastern Pacific Ocean and Hawaii, Vol. N,* E. L. Winterer, D. M. Hussong, and R. W. Decke, eds. pp. 188-217. Geological Society of America, New York, New York.

Emerson, W. K. and H. W. Chaney. 1995. A zoogeographic review of the Cypraeidae (Mollusca: Gastropoda) occurring in the eastern Pacific Ocean. *The Veliger* 38:8-21.

Govan, H. 1994. Cymatium muricinum *and Other Ranellid Gastropods: Major Predators of Maricultured Tridacnid Clams.* Doctoral dissertation, Heriot-Watt University, Edinburgh. xiii + 121 pp.

Kay, E. A. 1971. The littoral marine molluscs of Fanning Island. *Pacific Science* 25: 260-281.

Kay, E. A. 1995. Evolutionary radiation in the Cypraeidae. *In: Origin and Evolutionary Radiation in the Mollusca,* J. D. Taylor, ed. pp. 211-220. Oxford University Press, Oxford.

Kay, E. A. and S. R. Palumbi. 1987. Endemism and evolution in Hawaiian marine invertebrates. *Trends in Ecology and Evolution* 2:183-186.

Kempf, S. C. 1981. Long-lived larvae of the gastropod *Aplysia juliana:* do they disperse and metamorphose or just slowly fade away? *Marine Ecology Progress Series* 6:61-65.

Knauss, J. A. 1970. Measurements of the Cromwell Current. *Deep-Sea Research* 6:265-286.

Lewontin, R. C. 1974. *The Genetic Basis of Evolutionary Change.* Columbia University Press, New York. xii + 346 pp.

Lobel, P. S. 1989. Ocean current variability and the spawning season of Hawaiian reef fishes. *Environmental Biology of Fishes* 24:161-171.

Lobel, P. S. and A. R. Robinson. 1986. Transport and entrapment of fish larvae by ocean mesoscale eddies and currents in Hawaiian waters. *Deep-Sea Research* 33:483-500.

Lobel, P. S. and A. R. Robinson. 1988. Larval fishes and zooplankton in a cyclonic eddy in Hawaiian waters. *Journal of Plankton Research* 10:1209-1223.

McNally, G. H., W. C. Patzert, A. D. Kirwan, and A. C. Vastano. 1983. The near surface circulation of the north Pacific using satellite tracked drifting buoys. *Journal of Geophysical Research* 88:7507-7518.

Mitton, J. B., C. B. Berg, and K. S. Orr. 1989. Population structure, larval dispersal and gene flow in the queen conch *Stombus gigas* of the Caribbean. *Biological Bulletin* 177:356-362.

Patzert, W. C. 1969. *Eddies in Hawaiian Waters.* Hawaii Institute of Geophysics, University of Hawaii, Technical Report H1G-69-8, 51 pp., 71 figs.

Paulay, G. 1989. Marine invertebrates of the Pitcairn Islands: species composition and biogeography of corals, molluscs and echinoderms. *Atoll Research Bulletin* 326: 28 pp.

Richmond, R. H. 1990. The effects of El Niño/southern oscillation on the dispersal of corals and other marine organisms. *In: Global Ecological Consequences of the 1982-1983 El Niño/Southern Oscillation,* P. W. Glynn, ed. pp. 127-140. Elsevier Oceanographic Series, Amsterdam.

Robinson, A. R. and P. S. Lobel. 1985. Impact of ocean eddies on coastal currents. *In: Hawaiian Ocean Experiment,* L. Magaard, V. Gaynor, and R. Pujalet, eds. pp. 323-334. Proceedings, Aka Hulikïa, Hawaiian Winter Workshop, Hawaii Institute Geophysics Special Report.

Scheltema, R. S. 1956. The effect of substrate on the length of planktonic existence in *Nassarius obsoletus. Biological Bulletin* 111:312.

Scheltema, R. S. 1961. Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biological Bulletin* 120:92-109.

Scheltema, R. S. 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow water benthic marine gastropods. *Biological Bulletin* 140:284-322.

Scheltema, R. S. 1978. On the relationship between dispersal of pelagic veliger larvae and the evolution of marine prosobranch gastropods. *In: Marine Organisms Genetics, Ecology and Evolution,* B. Battaglia and J. A. Beardmore, eds. pp. 303-322. Plenum Press, New York.

Scheltema, R. S. 1986. Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific Islands. *Bulletin of Marine Science* 39:241-256.

Scheltema, R. S. 1989. On the children of benthic invertebrates: their rambling and migration in time and space. *In: Environmental Quality and Ecosystem Stability, Vol. IVB, Environmental Quality,* E. Spanier, Y. Steinberger, and M. Luria, eds. pp. 93-112. Israel Society for Ecology and Environmental Quality Sciences, Jerusalem.

Scheltema, R. S. 1992. Passive dispersal of planktonic larvae and the biogeography of tropical sublittoral invertebrate species. *In: Marine Eutrophication and Population Dynamics,* G. Colombo, I. Ferari, V. U. Ceccherelli, and R. Rossi, eds. pp. 195-202. Olsen and Olsen, Fredensborg.

Scheltema, R. S. 1995. The relevance of passive dispersal for the biogeography of Caribbean mollusks. *American Malacological Bulletin* 11:99-115.

Scheltema, R. S. and I. P. Williams. 1983. Long-distance dispersal of planktonic larvae and the biogeography and evolution of some Polynesian and western Pacific mollusks. *Bulletin of Marine Science* 33:545-564.

Schilder, F. A. 1969. Zoogeographical studies on living cowries. *The Veliger* 11:367-377.

Taylor, J. B. 1975. *Planktonic Prosobranch Veligers of Kaneohe Bay.* Doctoral dissertation, University of Hawaii, Honolulu. xiii + 599 pp.

Tomczak, M. and J. S. Godfrey. 1994. *Regional Oceanography: An Introduction.* Pergamon Press, New York. vii + 422 pp.

Toole, J. M., E. Zou, and R. C. Millard. 1988. On the circulation of the upper waters in the western equatorial Pacific Ocean. *Deep-Sea Research* 35:1451-1482.

Walker, G. P. L. 1990. Geology and volcanology of the Hawaiian Islands. *Pacific Science* 44:315-347.

Wyrtki, K. 1966. Oceanography of the eastern equatorial Pacific Ocean. *Annual Review of Oceanography and Marine Biology* 4:33-68.

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SYMPOSIUM: EVOLUTION OF COLEOID CEPHALOPODS

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Biochemical and molecular approach to cephalopod phylogeny

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Abstract: Cephalopod taxonomy is still uncertain, and little is known of the phylogeny of Recent taxa. Biochemical and molecular characters are complementary to morphology, and allow an additional insight into the phylogenetic relationships among cephalopods. Eye lens protein electrophoresis and immunological approaches yield data in agreement with traditional taxonomic grouping, but are less suitable for establishing phylogenetic relationships. Molecular tools, *e. g.* the 3' end of the 16S rDNA gene, have failed to resolve the phylogeny at the suprafamilial level, but seem appropriate at lower levels. DNA sequence comparisons (% substitution) show that a direct relationship between taxonomic rank and nucleotide divergence cannot be established, as the nucleotide divergence level differs from one taxa to the other.

Electrophoretic and immunological analyses of eye lens proteins as well as molecular results suggest that sepiolids should be separated from Sepioidea.

Phylogenetic analyses allow hypotheses of biological evolution based on various criteria to be tested. Morphological classification of taxa has, historically, been the first approach considered, and the analyses of anatomical structures are still of prime importance in the construction of hypotheses on the evolutionary history of organisms. But more recently, the development of biochemical and molecular techniques, that allow an insight into genetic structure, have opened new perspectives in taxonomy and phylogeny. Paleontology provides, however, the only direct means of calibrating evolutionary events. Both paleontology and embryology allow recognition of homologous characters derived from a common ancestor, the ancestors being deduced from the characters of terminal groups.

As far as cephalopods are concerned, the taxonomic status of many groups is still uncertain, and little is known of the phylogeny of Recent taxa. The main reason for this is that although the fossil record contains a wealth of ectocochleate forms (all extinct except *Nautilus*), this is not the case for Recent, mainly soft-bodied animals with a reduced or absent internal shell. So far only a few authors have considered the phylogenetic systematics of the group Cephalopoda. Fioroni (1981) was one of the first to use the Hennig (1966) approach to systematics. Berthold and Engeser (1987) established adelphotaxonomic relationships by the identification of synapomorphies in fossil and Recent taxa and proposed a phylogenetic classification of 35 subordinate taxa. The cladograms presented in these papers are not totally in agreement, drawing attention to the necessity of using additional criteria, such as biochemical and molecular, for taxonomic and phylogenetic reconstruction of cephalopod systematics.

All approaches, morphological, biochemical, and molecular, are complementary but each has advantages and disadvantages. Molecular analyses provide new tools to test the hypotheses based on morphology, and help to reformulate them in some cases. Morphological characters are more accessible, easier and less costly to analyze, allow comparison of extant and fossil forms, but can be more subjective. Biochemical and molecular characters are more objective, are potentially very abundant, but are not always easy to analyze. For instance, the fact that the nucleotides at each position can exist in only four states could be an important source of homoplasy. It is also well known that different portions of the same gene have not all the same probability of variation, and the rate of evolution can be different for the same gene in different taxa.

This paper is aimed to compare the results obtained using biochemical (electrophoretic and immunological) and molecular techniques for phylogenetic reconstruction of cephalopods using a number of species, comprising octopods and decapods.

MATERIALS AND METHODS

Fresh cephalopod tissue samples were obtained from various sources (Table 1). They were either stored at -20°C prior to electrophoretic and immunological analyses, or alcohol preserved for nucleotide sequencing.

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Table 1. List of cephalopod species analyzed by eye lens protein electrophoresis and mtDNA sequencing. Their geographical origin is indicated as well as the source of the data: 1, Bonnaud *et al,* 1994; 2, Tranvouez and Boucher-Rodoni, 1990; X, present paper; (-), no data.

Origin	Species	Eye lens	mtDNA
East Atlantic (Biscay)	*Sepia officinalis* Linné, 1758	X	1
Mediterranean (Banyuls)	*S. orbignyana* Férussac, 1826	2	1
SW Pacific (New Caledonia)	*S. latimanus* Quoy and Gaimard, 1832	X	1
English Channel (Roscoff)	*Loligo vulgaris* Lamarck, 1798	2	1
SW Pacific (New Caledonia)	*Sepioteuthis lessoniana* Lesson, 1830	X	1
English Channel (Roscoff)	*Sepiola atlantica* Orbigny, 1840	X	1
Mediterranean (Banyuls)	*Rossia macrosoma* (Delle Chiaje, 1829)	X	1
Mediterranean (Banyuls)	*Todaropsis* sp.	X	(–)
Pacific Ocean (Hawaii)	*Todarodes* sp.	(–)	1
Mediterranean (Banyuls)	*Octopus vulgaris* Cuvier, 1797	2	(–)
Mediterranean (Banyuls)	*Eledone cirrhosa* (Lamarck, 1798)	2	1
SW Pacific (New Caledonia)	*O. cyanea* Gray, 1849	X	1
SW Pacific (New Caledonia)	*O.* sp.	X	(–)
East Atlantic (Mauritania)	*Graneledone verrucosa* (Verrill, 1881)	X	(–)
East Atlantic (Mauritania)	*Opistoteuthis agassizii* (Verrill, 1883)	X	(–)

For biochemical approaches (electrophoresis and immunology), the protein chosen should be stable enough that individual physiological changes do not influence the observed differences between species, but it should be variable enough to reflect taxonomic and phylogenetic differences. Eye lens proteins and hemocyanin were tested here.

Eye lens proteins were extracted according to the protocols described in Tranvouez and Boucher-Rodoni (1990), and analyzed on precast polyacrylamide gels (ExcelGel SDS Gradient 8-18%, Pharmacia). A band presence/absence matrix was computed and processed by the NTSYS-pc program (Rohlf, 1990), using Sahn clustering (Sneath and Sokal, 1973) with UPGMA and Neighbor-Joining (NJ) methods (Saitou and Nei, 1987), to produce phenograms. PAUP 3.1 (Swofford, 1985) was used to estimate phylogenetic trees.

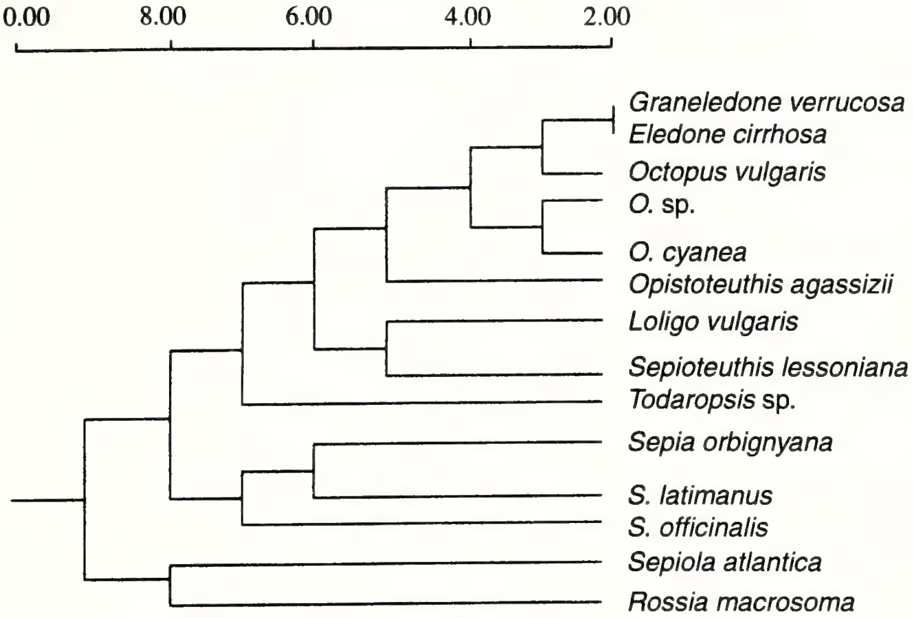
Protein antigenic properties are supposed to allow the estimation of immunological distance between taxa (Tsusumi *et al.*, 1989). The ELISA immunological technique, an enzyme-linked immunosorbent assay, was adapted to cephalopod eye lens protein (Boucher-Rodoni *et al.*, 1995). Four eye lens antisera were available (*Sepia officinalis, S. orbignyana, Loligo vulgaris, Octopus vulgaris*). A homologous standard inhibition curve was determined with 2500-fold diluted serum, and the affinity of heterologous samples was then tested.

The antigenic properties of hemocyanin, the large respiratory protein found in the blood of all cephalopods, was also used here to estimate taxonomic relationships. The immunological distance of various cephalopod species was estimated by heterologous reaction against commercial keyhole limpet hemocyanin (KLH) and compared to preliminary results obtained with homologous antiserum.

For molecular analyses, attention was first focused on mitochondrial DNA (mtDNA) because of its diversity, and because data on various groups, including non-molluscan invertebrates, are well known. mtDNA is a small circular DNA molecule present in many copies in the mitochondria, it is maternally inherited, and there is no recombination. The protein genes and the r-RNA genes have both a mosaic structure of conserved and variable regions, which should allow phylogenetic relationships at various hierarchical levels to be analyzed. Nucleotide sequence data from the 3' end of the 16S rDNA gene have already been used to analyze phylogenetic relationships among decapod cephalopods (Bonnaud *et al.*, 1994). To determine whether the different taxonomic hierarchical levels can be related to a given nucleotide percentage of divergence, some sequences from two populations of the same species, from different species of the same genus, and from different genera, were compared and analyzed in terms of molecular divergence (% substitution).

RESULTS

The results of eye lens protein electrophoresis of 14 cephalopod species analyzed by UPGMA and Neighbor-Joining methods show that with both methods the taxonomic grouping is respected (Fig. 1). Octopods are always grouped together, but are not distinctly separated from decapods. Incirrate octopods are a sister group of cirrate octopods, but at lower taxonomic levels the genus *Octopus* is not homogeneous. As far as decapods are concerned, the relationships among myopsids, oegopsids, and sepioids are not clearly defined by either approach. The sepiolids occupy a particular position in the NJ analysis, branching as a sister group of all other coleoids. The strict consensus tree derived from the phylogenetic analysis (PAUP 3.1) is less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Graneledone verrucosa
Eledone cirrhosa
Octopus vulgaris
O. sp.
O. cyanea
Opistoteuthis agassizii
Loligo vulgaris
Sepioteuthis lessoniana
Todaropsis sp.
Sepia orbignyana
S. latimanus
S. officinalis
Sepiola atlantica
Rossia macrosoma

Fig. 1. Phylogenetic tree obtained by the Neighbor-Joining method on SDS-Page electrophoretic analysis of eye lens proteins.

resolved for octopods (Fig. 2).

When using protein eye lens immunological properties to estimate immunological distances for phylogenetic analyses, one of the main problems encountered was that in some cases the distances were not symmetrical. Table 2 shows that when comparing the heterologous reaction between two species using each species extract in turn as antiserum and antigen, the immunological distance is not necessarily the same.

The hemocyanin is currently used as an immunogenic agent. In cephalopods it is composed of seven functional units in octopods and *Nautilus*, and eight functional units in decapods (Van Holde *et al.*, 1992). Fig. 3 shows the immunoreactivity of the hemocyanin of various cephalopod species against KLH antiserum, estimated by the ELISA technique. *Nautilus* reactivity was the closest to KLH homologous reaction, most of the other species being grouped together at a rather distant position, except *Sepia* which displayed a reactivity stronger than all other coleoids. Preliminary assays with homologous hemocyanin antiserum indicate that the distances are readable at high hierarchical levels (*i. e.* distant taxa), but do not discriminate species. The distance between *Sepia* and *Nautilus* was the smallest, but the difference was not as important as with KLH. And again, as with eye lens proteins, the results were not symmetrical.

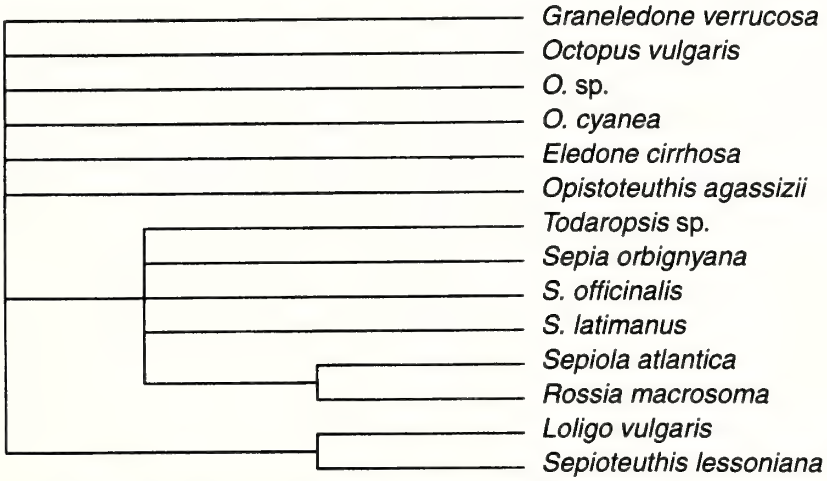
Graneledone verrucosa
Octopus vulgaris
O. sp.
O. cyanea
Eledone cirrhosa
Opistoteuthis agassizii
Todaropsis sp.
Sepia orbignyana
S. officinalis
S. latimanus
Sepiola atlantica
Rossia macrosoma
Loligo vulgaris
Sepioteuthis lessoniana

Fig. 2. Strict consensus tree (unrooted) of 100 trees obtained by heuristic search (PAUP 3.1; MULPARS option).

Table 2. Results of immunological heterologous reaction (ELISA technique) between species pairs using each species extract in turn as antiserum and antigen. Values represent immunological distances estimated by optical density differences.

ANTISERUM		ANTIGEN
Sepia officinalis	$\overset{4}{\underset{4}{\leftrightarrows}}$	*S. orbignyana*
S. officinalis	$\overset{10}{\underset{15}{\leftrightarrows}}$	*Loligo vulgaris*
L. vulgaris	$\overset{17}{\underset{13}{\leftrightarrows}}$	*S. orbignyana*
Octopus vulgaris	$\overset{16}{\underset{25}{\leftrightarrows}}$	*S. officinalis*
O. vulgaris	$\overset{15}{\underset{17}{\leftrightarrows}}$	*S. orbignyana*
O. vulgaris	$\overset{24}{\underset{25}{\leftrightarrows}}$	*L. vulgaris*

To analyze the relationships of cephalopods at a perispecific level (population, species), many authors have studied enzymatic polymorphism which remains a valuable tool for genetic population analyses (Levy *et al.*, 1988; Carvalho *et al.*, 1992; Brierley *et al.*, 1993, 1995).

If mtDNA is considered, preliminary results on six different populations of *Sepia officinalis* indicate that neither 16S, nor cytochrome oxidase CoII or CoIII, displays adequate variability for taxonomic purposes, whereas enzymatic polymorphism does (Bonnaud, unpub. data). Accordingly, when comparing the 3' end of 16S rDNA gene sequences, in terms of % substitution, the difference between two distant populations of *S. officinalis* (Mediterranean and English Channel) is not significant (Table 3). Therefore, this gene portion is not appropriate to provide evidence of differences among *S. officinalis* populations.

At higher taxonomic levels, the status of many cephalopod groups is still uncertain and needs to be reconsidered with the help of phylogenetic reconstruction. Phylogenetic hypotheses to estimate divergence time are usually given through paleontology (Doyle *et al.*, 1994). True cuttlebones are only known from the early Tertiary; Teuthoidea and Sepioidea are thus supposed to have diverged in the Cenozoic.

The use of mtDNA 16S for phylogenetic reconstruction was shown to be appropriate to test Tertiary divergences in insects and vertebrates (Simon *et al.*, 1990; Hillis and Dixon, 1991), but such is not the case for cephalopods (Bonnaud *et al.*, 1994). The phylogeny of cephalopods is unresolved at the suprafamilial level because of excessive nucleotide divergence (saturation), perhaps due to earlier emergence than Cenozoic, or to unequal evolutionary rates among taxa.

To compare results derived from protein elec-

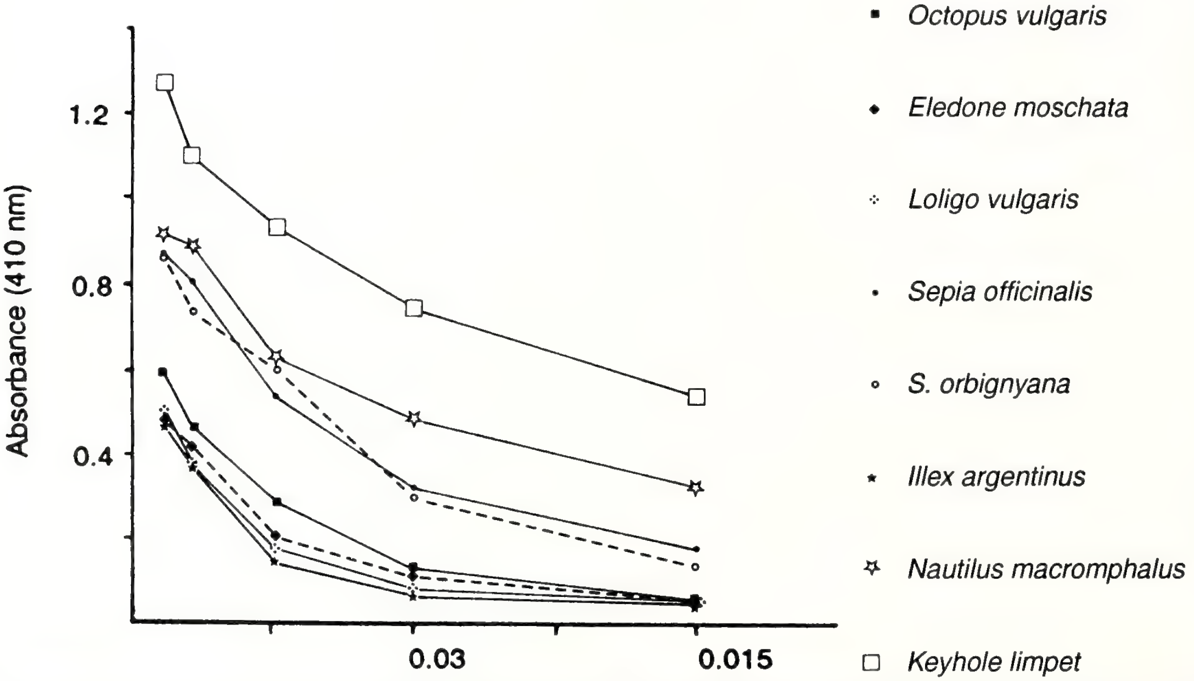

Fig. 3. Protein (µg/ml) Immunoreactivity of hemocyanin of various cephalopod species against keyhole limpet hemocyanin antiserum, tested by the ELISA technique.

Table 3. Nucleotide divergence of 3' end of 16S rDNA gene sequences at various taxonomic levels: (a) populations, (b) intrageneric, (c) intergeneric.

	Species	Divergence (% substitution + gaps)
(a)	*Sepia officinalis* (Roscoff) - *S. officinalis* (Banyuls)	*ca.* 1
(b)	*S. officinalis* - *S. orbignyana*	13.8
	S. orbignyana - *S. elegans*	8.1
	S. elliptica - *S. pharaonis*	10.3
	S. pharaonis - *S. smithi*	8.8
	Loligo forbesi - *L. vulgaris*	9.4
	Nautilus macromphalus - *N. pompilius*	5.5
(c)	*Sepietta* sp. - *Sepiola atlantica*	5.3
	Sepietta sp. - *Rossia macrosoma*	13.0
	Sepiola atlantica - *Rossia macrosoma*	12.1
	Sepietta sp. - *Sepia officinalis*	17.9
	Sepietta sp. - Loligo vulgaris	18.4*

*Overestimated value, because of an insertion of *ca.* 20 bases in *Loligo*.

trophoresis and sequence data from the 3' end of 16S rDNA, the sequences corresponding to some of the taxa appearing in Fig. 1 were aligned and analyzed by Neighbor-Joining and PAUP methods. Both approaches show that nucleotide analysis clearly separates octopods and decapods, but again the monophyly of the order Sepioidea, including sepiids and sepiolids is not supported, the sepiolids being excluded from the order (Fig. 4). In terms of % substitution, sequence comparison shows that sepiolids are as distant from *Sepia* (17.9%) as from *Loligo* (18.4%) (Table 3). Intrageneric divergence ranges from 8 to 14% for sepiids (the highest value concerning *S. officinalis* and *S. orbignyana*), but a direct relationship between taxonomic rank and nucleotide divergence cannot be established, as the nucleotide divergence level in other taxa could be in another range. Between sepiolid genera, for instance (Table 3), the intergeneric divergence is 5.3% between *Sepiola* and *Sepietta*, two morphologically closely related species, but can be as high as 13.0% between *Sepietta* and *Rossia*. One surprising result concerns the sequence comparison of two morphologically distinct species of *Nautilus* which display a low nucleotide divergence value (5.5%).

DISCUSSION AND CONCLUSION

The reliability of eye lens proteins as a taxonomic tool was shown by some authors (Smith, 1969; Swanborn, 1971; Brahma and Lancieri, 1979; Tranvouez and Boucher-Rodoni, 1990). It is confirmed here that eye lens protein electrophoresis analysis serves to group closely related taxa together, but its use for inferring phylogenetic relationships leads to more questionable results. The use of immunological properties of eye lens proteins and of hemocyanin to analyze taxonomic relationships is rather disappointing, mainly because the distances measured by the ELISA technique between taxa are not symetrical, *i. e.* the distance between *Sepia* and *Nautilus* is not the same as the distance between *Nautilus* and *Sepia*. The immunological distance indicates the degree of similarity, but it is not a character that is easy to precisely quantify for phenetic or phylogenetic analyses.

The development of molecular biology has raised great hope and excitement about phylogenetic reconstructions. We now have direct access to the genetic material. However the enormity of the available genetic information is itself a problem: where is the most appropriate place to investigate to answer our questions? Rates of evolution differ from one gene to the other, and the main problem is to find a genetic marker appropriate for the hierarchical level we are interested in. If a gene has evolved too rapidly, it will be saturated with substitutions and provide a non-significant result. If it is too stable, the variability will not be informative enough. Our knowledge of genetic structures comes primarily from results obtained with vertebrates or *Drosophila*, but their levels of nucleotide divergence are not necessarily adequate for analysis of other phylogenetic relationships. This cannot be known *a priori*, and a series of prerequisites are necessary before starting a molecular analysis. (1) Choice of the gene: its variability must be adequate to the hierarchical level being considered. (2) Choice of the species (number and "quality"): this is important because some cephalopod genera include over 100 species, whereas others are monotypic. (3) Length of the sequence: apparently, bootstrap values increase with length of sequence, but the reliability depends rather on the number of taxa (Lecointre *et al.*, 1993). So, as with morphology, we will have to increase, as much as possible, the number of species for each taxon. This is obviously impossible when some genera or even families are monospecific. (4) Choice of an outgroup: it should comprise more than one species of a monophyletic group, close enough to the investigated taxon to preclude saturation, but sufficiently different to prevent inclusion in the ingroup.

As far as cephalopods are concerned, nucleotide sequence analysis provides a more reliable picture than electrophoresis and immunology which are interesting taxonomic tools, but are not satisfactory for phylogenetic analyses. However, a direct relationship between taxonomic rank and % nucleotide divergence cannot be established, as the nucleotide divergence level is different in different taxa.

The congruence between morphological and molecular analyses, two independent sets of data, is very important in construction of evolutionary patterns. Morphological-molecular comparisons however are still rare, and

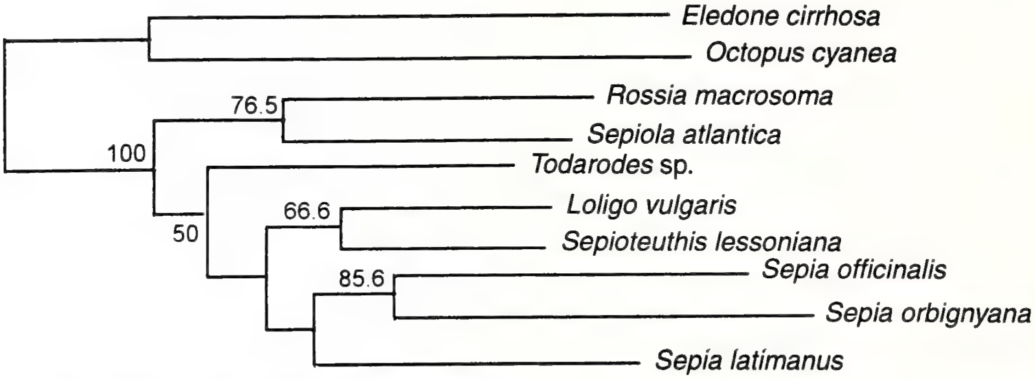

Fig. 4. Phylogeny inferred by Neighbor-Joining distance analysis from mtDNA 16S sequences (Bonnaud *et al*, 1994). Bootstrap values ≤ 50 are indicated.

not often congruent. In cephalopods, one solid and congruent result concerns sepiolids. Their taxonomic and phylogenetic position has been a matter of much discussion, being classified either as a family of the Sepioidea (Naef, 1912; Voss, 1977; Mangold and Portman, 1989) or as a family of the Myopsida together with the Sepiidae (Berthold and Engeser, 1987). Fioroni (1981, based on embryology) and Clarke (1988, based on morphology) proposed to raise the sepiolids to ordinal rank. The present electrophoretic, immunological, and molecular results confirm that sepiolids can be separated from the Sepioidea.

The analysis of phylogenetic relationships among coleoids must take into account a number of difficulties, whatever the criteria used, morphological, biochemical, or molecular: (1) poor fossil remains of recent taxa; (2) no outgroup: we choose octopods as an outgroup for decapods, and *vice versa* because, even if the distance is very important, we have no real alternative; (3) many monospecific genera; (4) traditional classification not well stabilized; (5) little information on rate and modalities of molecular evolution: the evolutionary rate of the different genes is inferred mainly from vertebrate results - vertebrates represent only one episode of the saga of life and it is not always possible to transpose to invertebrates genetic postulates based on vertebrate results.

To better understand higher hierarchical levels of phylogeny, it appears necessary in many cases to consider more than one gene, preferably by associating mitochondrial and nuclear information.

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LITERATURE CITED

Berthold, T. and T. Engeser. 1987. Phylogenetic analysis and systematization of the Cephalopoda (Mollusca). *Verhandlungen des naturwissenschaftlichen Vereins Hamburg* (NF) 29:187-220.

Bonnaud, L., R. Boucher-Rodoni, and M. Monnerot. 1994. Phylogeny of decapod cephalopods based on partial 16S rDNA nucleotide sequences. *Comptes Rendus de l'Académie des Sciences, Paris, Sciences de la Vie* 317:581-588.

Boucher-Rodoni, R., M. Martin, and R. Peduzzi. 1995. Preliminary results of a comparative study on antigenicity of cephalopod eye-lens proteins: the sepiolids. *Bulletin de l'Institut Océanographique, Monaco* 16:93-98.

Brahma, S. K. and M. Lancieri. 1979. Isofocusing and immunological investigations on cephalopods lens proteins. *Experimental Eye Research* 29:671-678.

Brierley, A. S., P. G. Rodhouse, J. P. Thorpe, and M. R. Clarke. 1993. Genetic evidence of population heterogeneity and cryptic speciation in the ommastrephid squid *Martialia hyadesi* from the Patagonian shelf and Antarctic Polar frontal zone. *Marine Biology* 116:593-602.

Brierley, A. S., J. P. Thorpe, G. J. Pierce, M. R. Clarke, and P. R. Boyle. 1995. Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the northeast Atlantic Ocean. *Marine Biology* 122:79-86.

Carvalho, G. R., A. Thompson, and A. L. Stoner. 1992. Genetic diversity and population differentiation of the shortfin *Illex argentinus. Journal of Experimental Marine Biology and Ecology* 158:105-121.

Clarke, M. R. 1988. Evolution of buoyancy and locomotion in recent cephalopods. *In: The Mollusca. Vol. 12, Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 203-213. Academic Press, New York.

Doyle, P., D. T. Donovan, and M. Nixon. 1994. Phylogeny and systematics of the Coleoidea. *University of Kansas Paleontological Contributions* 5:1-15.

Fioroni, P. 1981. Die Sonderstellung der Sepioliden, ein Vergleich der Ordnungen der rezenten Cephalopoden. *Zoologische Jahrbücher, Systematik* 108:178-228.

Hennig, W. 1966. *Phylogenetic Systematics.* University of Illinois Press, Urbana. 263 pp.

Hillis, D. M. and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Quarterly Review of Biology*

66(4):411-453.

Lecointre, G., H. Philippe, and H. Le Guyader. 1993. Species sampling has a major impact on phylogenetic inference. *Molecular Phylogenetics and Evolution* 2(3):205-224.

Levy, J. A., M. Haimovici, and M. Conceicao. 1988. Genetic evidence for two species of the genus *Eledone* (Cephalopoda: Octopodidae) in south Brazil. *Comparative Biochemistry and Physiology* 90B(2):275-277.

Mangold, K. and A. Portman. 1989. Systématique. *In: Traité de Zoologie*. P. P. Grassé, ed. pp. 643-713. Masson et cie, Editeurs, Paris.

Naef, A. 1912. Teuthologische Notizen. 2. Die Gattungen der Sepioliden. *Zoologischer Anzeiger* 39: 244-248.

Rohlf, F. J. 1990. *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System*. Exeter Software, New York.

Saitou, N. and M. Nei. 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Simon, C., S. Pääbo, T. D. Kocher, and A. C. Wilson. 1990. Evolution of mitochondrial ribosomal RNA in insects as shown by the polymerase chain reaction. *In: Molecular Evolution,* M. Clegg and S. O'Brien, eds. UCLA Symposia on Molecular and Cellular Biology, New Series, vol. 122. pp. 235-244. Wiley-Liss, New York.

Smith, A. C. 1969. An electrophoretic study of proteins extracted in distilled water and in saline solution, from the eye lens nucleus of the squid, *Nototodarus hawaiiensis* (Berry). *Comparative Biochemistry and Physiology* 30: 551-559.

Sneath, P. H. A. and R. R. Sokal. 1973. *Numerical Taxonomy*. Freeman, San Francisco. 573 pp.

Swanborn, P. L. 1971. Cephalopod lens proteins. *Experimental Eye Research* 11:111-115.

Swofford, D. L. 1985. *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1*. Illinois Natural History Survey, Champaign, Illinois.

Tranvouez, Y. and R. Boucher-Rodoni. 1990. Eye lens SDS-Page electrophoresis and the systematics of cephalopods. *Cahiers de Biologie Marine* 31:281-289.

Tsusumi, H., S. Nakamura, K. Sato, K. Tamaki, and Y. Katsumata. 1989. Comparative studies on an antigenicity of plasma proteins from humans and apes by ELISA: a close relationship of chimpanzee and human. *Comparative Biochemistry and Physiology* 94B:647-649.

Van Holde, K. E., K. I. Miller, and W. H. Lang. 1992. Molluscan hemocyanins: structure and function. *In: Advances in Comparative and Environmental Physiology,* Vol. 13. pp. 257-300. Springer Verlag, Berlin and Heidelberg.

Voss, G. L. 1977. Present status and new trends in cephalopod systematics. *In: The Biology of Cephalopods,* M. Nixon and J. B. Messenger, eds. pp. 49-60. Symposium of the Zoological Society of London, 38.

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Relationship of some coleoid cephalopods established by 3' end of the 16S rDNA and cytochrome oxidase III gene sequence comparison

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Abstract: Phylogenetic relationships for extant cephalopods have been based, so far, mainly on morphology and paleontology. Nucleotide sequence data are still rare. Sequence analyses from the 3' end of the 16S rDNA gene of cephalopods have shown that this portion of gene can provide valuable information on taxonomic relationships at the infrafamilial level. Another mitochondrial gene, cytochrome oxidase III, is investigated to analyze higher (*i. e.* ordinal) taxonomic levels. The results obtained by the two gene portions are compared, but the low number of species does not allow a definitive answer on interfamilial relationships. The low divergence between nucleotide sequences of two populations of *Loligo vulgaris* Lamarck, 1798, and of *L. reynaudii* Orbigny, 1845, suggests that the latter is not a clearly distinct species. The grouping of the three families of Sepioidea (Sepiidae, Spirulidae, and Sepiolidae) is not supported. Idiosepiidae groups with the oegopsid squid *Enoploteuthis* irregardless of the analysis (parsimony or distance).

The Decapoda are composed of two orders, Teuthoidea and Sepioidea. Relationships within these orders are not stabilized based on morphological characters. It is now possible with molecular characters to obtain a new type of information which can help to resolve some phylogenetic relationships. The 3' end of the mitochondrial- *l-r*-RNA (16S) was already investigated but with this portion of the molecule, extensive nucleotide variability leads to an unresolved phylogeny between the orders (Bonnaud *et al.*, 1994). Another mitochondrial gene, coding for cytochrome oxidase III (COIII), was chosen here to analyze the relationships between some species of decapods. The impact of mutations on protein function is very important, and accordingly, the structure of some genes coding for proteins should be less variable at the nucleotide level: this is the case for the genes coding for cytochrome oxidase subunits, COI, COII, and COIII. COIII gene analyses were thus thought to be suitable for solving phylogenetic relationships at hierarchical taxonomic levels higher than those resolved by the 16S gene. For this preliminary study, one species in each family or suborder was analyzed and the results obtained with the two gene portions compared.

MATERIAL AND METHODS

Details on the taxonomic position and origin of the eight species studied are presented in Table 1. DNA was extracted from frozen or alcohol-preserved tissues according to the protocol described in Bonnaud *et al.* (1994). A portion of 16S and a portion of COIII were amplified with universal primers: 984 and 986 for 16S and COIIIa and COIIIb according to the classification of Simon *et al.* (1991). These portions were cloned in pBS+ (Stratagène) and sequenced (ca. 500 pb each) with the dideoxy chain termination (Sanger *et al.*, 1977). The alignments were performed by eye, with the aid of secondary structure for 16S and of the reading coding frame for COIII. Phylogenetic trees were calculated by distance (Neighbor-Joining) method using the MUST package (Philippe, 1992) and parsimony method using PAUP 3.1 (Swofford, 1990). These two methods gave similar results and only the trees obtained with the distance method are described here. The robustness of internal branching was tested by bootstrapping.

Transversions (changes of pyrimidine to purine or *vice versa*) are known to be less abundant than transitions (changes of purine to purine or pyrimidine to pyrimidine) in some vertebrate taxa. When sequences are highly variable, transitions can introduce noise in the analyses. As a consequence, the use of transversions should lower the incidence of homoplasy between distant taxa. Analyses were performed using both all the substitutions and only the transversions. Attributing weight to the transversions instead of removing the transitions did not further change the results obtained.

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Table 1. Geographical origin and systematic position of species studied.

SPECIES (ORIGIN)	SYSTEMATIC POSITION	
Sepia officinalis Linné, 1758 (Banyuls)	Sepiidae	SEPIOIDEA
Sepietta sp. (Banyuls)	Sepiolidae	
Spirula spirula (Linné, 1758) (New Caledonia)	Spirulidae	
Idiosepius pygmaeus Steenstrup, 1881 (Australia)	Idiosepiidae	
Enoploteuthis reticulata Rancurel, 1970 (Hawaii)	Enoploteuthidae (Oegopsid squid)	TEUTHOIDEA
Loligo vulgaris Lamarck, 1798 (Roscoff)	Loliginidae (Myopsid squids)	
L. vulgaris (Banyuls)		
L. reynaudii Orbigny, 1845 (South Africa)		
Octopus cyanea Gray, 1849 (New Caledonia)	Octopodidae	

RESULTS

Analysis of partial 16S gene

Analyses were performed with 131 informative sites out of 231 variable sites. In agreement with the results obtained previously (Bonnaud *et al.*, 1994) the tree issued from the analysis of *l-r*-RNA gene is unresolved (Fig. 1). No strong relationship can be established among taxa, except for the Loliginidae. This is correlated with the sequence identities: the sequences of the two *Loligo vulgaris* populations are identical and that of *L. reynaudii* differs by only 1.1%, a percentage close to the error percentage generally accepted after amplification, cloning, or sequencing. The general topology of the tree appears coherent with the classification issued from morphological data (*i. e. Idiosepius, Sepia,* and *Sepietta* grouped together) but none of the groupings is strongly supported by a high bootstrap value. *Idiosepius* is not more closely related to the Sepioidea than to the Teuthoidea. It must be stressed that a complementary analysis with all available species did not provide a clearer answer, the substitutions between families being saturated (Bonnaud *et al.*, 1994): *Idiosepius'* position cannot be determined.

Analysis of partial COIII gene

Analyses were performed with 166 informative sites out of 258 variable sites of a portion of cytochrome oxidase III gene. The trees obtained with nucleotide sequence analysis (Figs. 2-3) likewise show a solid grouping of the three loliginids. The sequences of the two populations of *Loligo vulgaris* differ significantly with this gene (4.9% of nucleotide divergence), and the divergence between *L. vulgaris* "Roscoff" and *L. reynaudii* was 6.3%. Their grouping was always supported by a very high bootstrap value (100).

Another well-supported group is composed of the oegopsid squid *Enoploteuthis reticulata* and of *Idiosepius pygmaeus*, the representative of Idiosepiidae, one of the sepioid families. This same grouping of *Idiosepius* and *Enoploteuthis* was obtained when taking all the substitutions into account or only the transversions. Grouping of the three other families of Sepioidea (Sepiidae, Spirulidae, and Sepiolidae) is not supported. This was confirmed by the separation of these three species in the PAUP analyses including all the substitutions or with weighted transversions (data not shown). When taking into account only the transversions, *Spirula* became linked with *Loligo* with a bootstrap value of 57.9 with Neighbor-Joining as well as with PAUP analyses, and the group excluding *Sepietta* and *Sepia* was well supported.

DISCUSSION AND CONCLUSION

It is clear that the two *Loligo* species are difficult to distinguish in terms of nucleotide variability with the

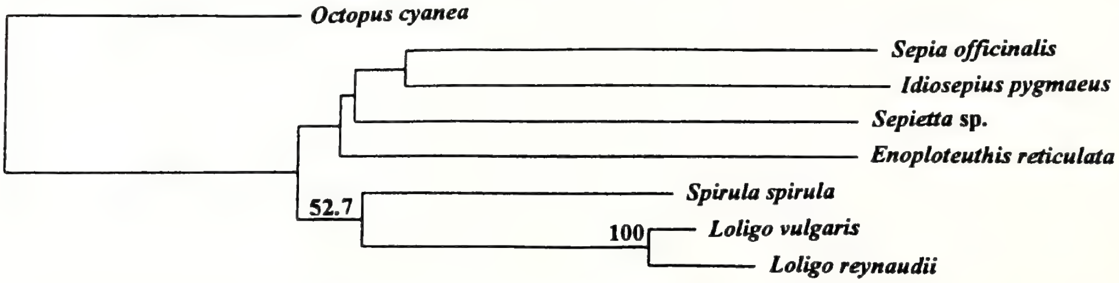
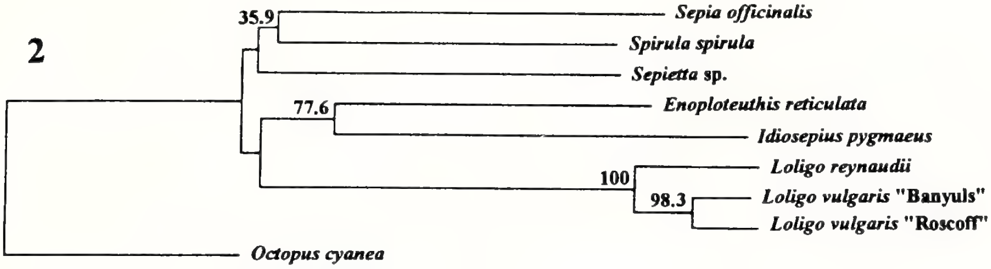
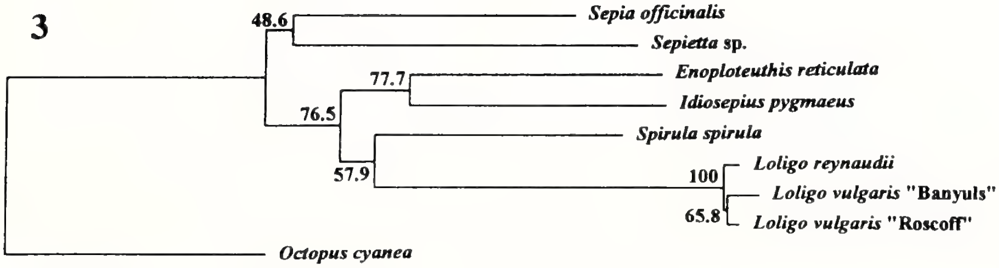


Fig. 1. Phylogenetic tree from the analysis of the 3' end of *l-r*-RNA using all substitutions (Neighbor-Joining method).

2


35.9
77.6
100
98.3
Sepia officinalis
Spirula spirula
Sepietta sp.
Enoploteuthis reticulata
Idiosepius pygmaeus
Loligo reynaudii
Loligo vulgaris "Banyuls"
Loligo vulgaris "Roscoff"
Octopus cyanea

3


48.6
77.7
76.5
57.9
100
65.8
Sepia officinalis
Sepietta sp.
Enoploteuthis reticulata
Idiosepius pygmaeus
Spirula spirula
Loligo reynaudii
Loligo vulgaris "Banyuls"
Loligo vulgaris "Roscoff"
Octopus cyanea

Figs. 2-3. Phylogenetic trees from analyses of partial COIII gene using (2) all the substitutions, (3) only the transversions (Neighbor-Joining method).

portions of the two mitochondrial genes. The observed divergence suggests differences due to geographical separation rather than a well-established speciation event. A study involving numerous specimens from along the French and African coasts would certainly provide an answer to this hypothesis of clinal variation.

The analysis of transversions only must be viewed with precaution, especially when the group excluding *Sepietta* and *Sepia* is considered. It is clear that the number of species was limited. In PAUP analyses of the COIII portion, *Sepietta* was linked with *Idiosepius* and *Enoploteuthis* whatever substitutions were considered. The opposing results from the two methods for the grouping of *Sepietta* reveal that this relation is uncertain and needs confirmation. The same is true for *Spirula* grouped with *Loligo*. The bootstrap values of 52.7 (with 16S) and 57.9 (with COIII) are low. If these values are really significant, they could be modified by increasing the species sampling, when possible.

On the contrary, *Idiosepius pygmaeus* is always grouped with the oegopsid irregardless of the analysis (parsimony or distance). This was unexpected because Idiosepiidae was placed by Naef (1916) with the Sepiidae and Sepiolidae as members of the order Sepioidea. For most authors, *Idiosepius* is more closely related to the Sepiolidae and Sepiadariidae than to the Sepiidae or Spirulidae, and its phylogenetic position has been questioned so far only with regard to the first two families (Fig. 4). The taxonomic rank of the idiosepiids has rarely been changed: it was always considered as a family of the order Sepioidea except by Guerra (1992) who raised Idiosepiidae to ordinal rank. The idiosepiids are isolated by characters like a dorsal adhesive organ in adults, the retardation of the tentacle development in juveniles, and the statocyst structure. They were often described without shell or gladius. The presence of a gladius was certainly difficult to detect because of the very small size of the members of this genus (20 mm maximum mantle length). Hylleberg and Nateewathana (1991a, b) found a thin gladius in the specimens examined and suggested that *I. pygmaeus* might be more closely related to Teuthoidea than to Sepioidea. Steenstrup (1881) created the genus *Idiosepius*; in his original description he mentioned that some specimens of small squids were described by early authors (*e. g.* Lamarck, Orbigny, Férussac, Blainville, Péron, Lesueur) under various names: *Cranchia minima* Férussac, 1835, *Loligo minima* Orbigny, 1848, *Loligopsis peronii* Lamarck, 1822, *Loligo parvula* Péron in Blainville, 1823, *Sepiola minima* Lesueur, 1821. In the opinion of Steenstrup, these decapods might be idiosepiids. The reasons which lead these early authors to attribute squid characteristics to *Idiosepius* could be an indication of the peculiar position of this genus within Decapoda. It is difficult to find morphological criteria which justify linking *Idiosepius* with sepiolids or sepiids and the few existing morphological studies, like those of Hylleberg and Nateewathana (1991a, b), do not analyze the taxonomic status of *Idiosepius*.

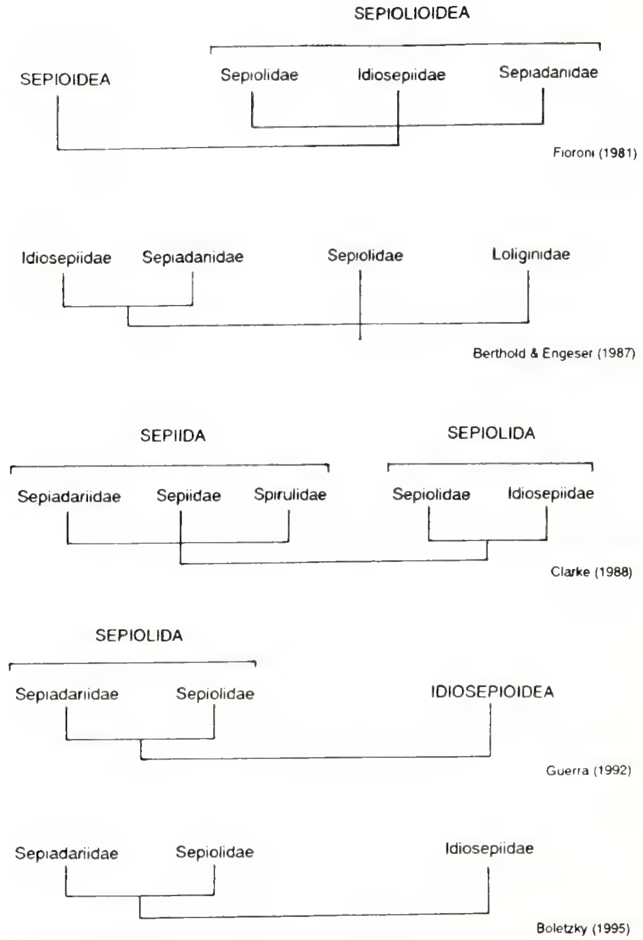

SEPIOLIOIDEA
SEPIOIDEA
Sepiolidae
Idiosepiidae
Sepiadanidae
Fioroni (1981)
Idiosepiidae
Sepiadanidae
Sepiolidae
Loliginidae
Berthold & Engeser (1987)
SEPIIDA
SEPIOLIDA
Sepiadariidae
Sepiidae
Spirulidae
Sepiolidae
Idiosepiidae
Clarke (1988)
SEPIOLIDA
Sepiadariidae
Sepiolidae
IDIOSEPIOIDEA
Guerra (1992)
Sepiadariidae
Sepiolidae
Idiosepiidae
Boletzky (1995)

Fig. 4. Relationships of idiosepiids with other taxa according to various authors. Ordinal rank indicated by capital letters.

Analysis of additional species, and especially of other oegopsid families, might help to confirm the unexpected position of *I. pygmaeus*, and eventually to relate it more closely to an oegopsid family (Bonnaud *et al.*, in press).

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LITERATURE CITED

Berthold, T. and T. Engeser. 1987. Phylogenetic analysis and systematization of the Cephalopoda (Mollusca). *Verhandlungen des naturwissenschaftlichen Vereins Hamburg* (NF) 29:187-220.

Boletzky S. v. 1995. The systematic position of the Sepiolidae (Mollusca: Cephalopoda). *Bulletin de l'Institut Oceanographique, Monaco* 16:99-104.

Bonnaud L., R. Boucher-Rodoni, and M. Monnerot. 1994. Phylogeny of decapod cephalopods based on partial 16S rDNA nucleotide sequences. *Comptes Rendus de l'Académie des Sciences, Paris, Sciences de la Vie* 317:581-588.

Bonnaud, L., R. Boucher-Rodoni, and M. Monnerot. In press. Phylogeny of cephalopods inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution.*

Clarke, M. R. 1988. Evolution of recent cephalopods. A brief review. *In: The Mollusca. 12. Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 331-339. Academic Press, London.

Fioroni, P. 1981. Die Sonderstellung der Sepioliden, ein Vergleich der Ordnungen der rezenten Cephalopoden. *Zoologische Jahrbücher, Systematik* 108:178-228.

Guerra, A. 1992. Mollusca, Cephalopoda. *In: Fauna Iberica, Vol. I,* M. A. Ramos *et al.*, eds. Museo Nacional de Ciencias Naturales, CSIC, Madrid. 327 pp.

Hylleberg, J. and A. Nateewathana. 1991a. Redescription of *Idiosepius pygmaeus* Steenstrup, 1881 (Cephalopoda: Idiosepiidae), with mention of additional morphological characters. *Phuket Marine Biological Center, Research Bulletin* 55:33-42.

Hylleberg, J. and A. Nateewathana. 1991b. Morphology, internal anatomy, and biometrics of the cephalopod *Idiosepius pygmaeus* Voss, 1962. A new record for the Andaman sea. *Phuket Marine Biological Center, Research Bulletin* 56:1-9.

Naef, A. 1916. Über neue Sepioliden aus dem Golf von Neapel. *Pubblicazioni della Stazione zoologica di Napoli* 1:1-10.

Philippe, H. 1992. MUST: a computer package of management utilitarians for sequences and trees. *Molecular Biology and Evolution* 4:406-425.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences* 74:5436-67.

Simon, C., A. Franke, and A. Martin. 1991. The polymerase chain reaction: DNA extraction and amplification. *In: Molecular Techniques in Taxonomy,* G. M. Hewitt, A. W. B. Johnston, and J. P. W. Young, eds. pp. 329-355. NATO Advanced Studies Institute, H57. Springer, Berlin.

Swofford, D. L. 1990. *PAUP: Phylogenetic Analysis Using Parsimony. Version 3.1.* Illinois Natural History Survey, Champaign, Illinois.

Steenstrup, J. 1881. Sepiadarium og *Idiosepius,* to nye slägter af sepiernes familie. *Danske Videnskabelig Selskap Skrifter* (6)1:212-242.

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Analysis of morphology to determine primary sister-taxon relationships within coleoid cephalopods

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Abstract: Although most families of living coleoid cephalopods are well defined, phylogenetic relationships among them are controversial. A necessary first step toward analyzing the phylogeny of decapod families is the determination of proper outgroups to polarize characters. The cladistic position of the Vampyromorpha is of particular interest. Toward this goal, we have examined 50 morphological characters in 24 species from 17 families. The material examined included representatives from the oegopsids, myopsids, sepioids and sepiolids, cirrate and incirrate octopods, and *Vampyroteuthis*. At this level, the characters were polarized either by comparison with *Nautilus*, or, for a few, by ontogenetic sequence or the fossil record. We found that of these 50 characters, 25 could not be used with confidence because of problems primarily involving character independence, apomorphic "loss," or assessment of homology/homoplasy. The states of three characters could be assumed to be ordered. Only ten characters were unambiguously informative as defining synapomorphies at the ordinal level. The resulting consensus of most-parsimonious trees is: (((oegopsid + myopsid + sepioid + sepiolid)(((cirrate)(incirrate)) vampire))(nautilus)).

Although most families of living coleoid cephalopods are well defined, phylogenetic relationships among them are controversial. Our understanding of cephalopod phylogeny is based mostly on the work of Naef (1921-1923; 1928). Advances in cephalopod systematics since the 1920s have mainly described new orders, families, genera, and species. The unraveling of the evolutionary pathways of this group has remained nearly stagnant. The phylogenetic classification presented by Voss (1977), summarized from much earlier authors, has been widely followed by most workers studying extant cephalopods (*e. g.* Roper *et al.*, 1984; Nesis, 1987; Mangold and Portmann, 1989; and the many researchers who have used their works). Many paleontologists, however, have not been comfortable with this classification. Relationships among the decapods have also been questioned (*e. g.* J. Z. Young, 1977; Fioroni, 1981; Boletzky, 1993a). Recently, Berthold and Engeser (1987) performed a phylogenetic analysis of the coleoid cephalopods and the resulting classification was quite different from that given by Voss. Perhaps the most important difference is that the myopsid squids (Loliginidae and Pickfordiateuthidae) were found to be more closely related to the cuttlefishes than they are to the other squids. Other recent attempts based on limited sets of characters (*e. g.* Clarke and Maddock, 1988; Clarke, 1988; Khromov, 1990) have not produced a credible genealogy of coleoid cephalopods.

The fossil record of coleoid cephalopods is generally meager with the exception of the hard structures of belemnites. Indeed, Donovan (1977: 45) concluded that "The phylogeny of living coleoids has to be compiled from hopelessly inadequate paleontological evidence. It is not surprising that attempts to work it out have been made at long intervals and have been more or less unconvincing." Much undescribed and unexamined fossil "teuthoid" material exists in museums (Donovan, 1977). Perhaps sufficient material exists that a convincing case for relationships could be made if interpreted within the general framework of a genealogy based on the analysis of Recent cephalopods.

Our virtual lack of progress in understanding cephalopod evolution is due to the fact that: (1) no broad-based morphological study has been attempted since Naef's work, although several dissertations on the comparative morphology of particular structures have produced valuable information (*e. g.* Toll, 1982, teuthoid gladius; Brakoniecki, 1986, loliginid hectocotylus; Hess, 1987, spermatophores; S. Candela, University of Miami, in preparation, beaks); (2) only a single molecular study of higher-level systematics has been published (Bonnaud *et al.*, 1994), and its results demonstrated the difficulty in selecting proper genes for analysis; and (3) studies using cladistic techniques have been few and these have dealt with genera within a family (*e. g.* Voss and Voss, 1983) or between just a few families

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(R. E. Young and Harman, in press). Cladistic techniques on a broad basis have not been properly used by either neontologists or paleontologists.

As a first-step toward analyzing cephalopod phylogeny we have chosen a top-down approach in order to determine outgroups for polarizing characters at lower phylogenetic levels. Our goals in this paper are: (1) to determine if the major coleoid taxa, Cirrata, Incirrata and Decapoda, are monophyletic (the monophyly of the Vampyromorpha is already established by its monotypy); and (2) to determine the relationships among these four taxa. Achievement of these goals will determine the placement of the Vampyromorpha and whether or not the Octopoda is monophyletic. We have examined 50 morphological characters in 24 species from 17 families to determine these ordinal and subordinal relationships. The material examined included representatives from the oegopsids, myopsids, sepioids and sepiolids, cirrate and incirrate octopods, and *Vampyroteuthis.*

Table 1. Species examined for all characters. ML = mantle depth.

FAMILY (HIGHER TAXA) Species	USNM Catalog No.	ML	Sex
BATHYTEUTHIDAE (OEGOPSIDA)			
Bathyteuthis abyssicola Hoyle, 1885	885673	63	female
	577804	40	male
	577804	53	female
BOLITAENIDAE (INCIRRATA)			
Japetella diaphana Hoyle, 1885	885674	41	juv.
	575636	80	female
J. heathi (Berry, 1911)	813756	40	juv.
ENOPLOTEUTHIDAE (OEGOPSIDA)			
Abralia trigonura Berry, 1913	730630	41	female
	730630	35	male
Enoploteuthis anapsis Roper, 1964	728753	62	male
	728753	81	male
	728754	72	female
	728754	40	female
GONATIDAE (OEGOPSIDA)			
Gonatus antarcticus Lönnberg, 1898	885675	139	female
LOLIGINIDAE (MYOPSIDA)			
Loligo pealei Lesueur, 1821	814246	160	male
Lolliguncula brevis (Blainville, 1823)	729175	104	female
Sepioteuthis sepioidea (Blainville, 1823)	814383	140	female
NAUTILIDAE (NAUTILOIDEA)			
Nautilus pompilius Linné, 1758	678868	70	female?
OCTOPODIDAE (INCIRRATA)			
Octopus vulgaris Cuvier, 1797	577100	48	female
OCYTHOIDAE (INCIRRATA)			
Ocythoe tuberculata Rafinesque, 1814	727831	62	female
OMMASTREPHIDAE (OEGOPSIDA)			
Illex illecebrosus (Lesueur, 1821)	885676	115	male
	885676	128	juv.
Ommastrephes bartramii (Lesueur, 1821)	814773	109	juv.
	814773	113	---
	814773	111	male?
ONYCHOTEUTHIDAE (OEGOPSIDA)			
Onychoteuthis banksii (Leach, 1817)	727524	93	female
OPISTHOTEUTHIDAE (CIRRATA)			
Opisthoteuthis agassizi Verrill, 1883	817405	27	female
O. californiana Berry, 1949	575640	31	female
SEPIIDAE (SEPIOIDEA)			
Sepia officinalis Linné, 1758	817479	84	male
SEPIOLIDAE (SEPIOIDEA)			
Rossia pacifica Berry, 1911	214611	33	female
Sepiola atlantica Orbigny, 1839-1842	575463	16	female
	575463	15	male
SPIRULIDAE (SEPIOIDEA)			
Spirual spirula (Linné, 1758)	814005	42	female
STAUROTEUTHIDAE (CIRRATA)			
Stauroteuthis syrtensis Verrill, 1879	817381	54	female?
	817383	50	not det.
THYSANOTEUTHIDAE (OEGOPSIDA)			
Thysanoteuthis rhombus Troschel, 1857	730192	147	male
VAMPYROTEUTHIDAE (VAMPYROMORPHA)			
Vampyroteuthis infernalis Chun, 1903	885677	55	female

MATERIALS AND METHODS

As terminal taxa for this study, we selected 17 families which we felt were representative of the putative major groups of extant coleoids although we did not include families that have highly derived autapomorphies (*e. g.* the coelomic specializations of cranchiid squids). Most families, other than monotypic families, were represented by two species often in two genera (Table 1). These species were examined for each character from specimens in the USNM collections (National Museum of Natural History, Washington, D. C.). In addition, a large variety of individuals was examined to determine the structure of a character in order to refine the character definition.

We surveyed 50 characters for each of the terminal taxa. The characters and their states are presented below in the Results section. Almost all character states were assessed by direct examination of specimens. In a few cases, such as neuroanatomy of the brain, we accepted reliable observations from the literature. As discussed below, half of the characters were eliminated from final analyses because of questions about independence, problems with assessing homology versus homoplasy, or our inability to define and assess unambiguous character states.

Nautilus is the clear outgroup for the coleoids. However, because *Nautilus* is so far removed from the coleoids morphologically, many of the ingroup characters are not applicable to it. In order to incorporate information from paleontology and ontogeny for polarity determination, we designated the outgroup as *Nautilus*/ancestral-coleoid. Thus, when a character was not applicable to *Nautilus* but could be polarized by fossil or developmental observations, these states were entered. When a character was not applicable to *Nautilus* and fossil/ontogenetic information was lacking, a "?" was entered in the data matrix.

Cladistic analyses were calculated using PAUP, version 3.1.1 (Swofford, 1993) and checked with Hennig86, version 1.5 (Farris, 1988) because the two programs treat data slightly differently in some circumstances (Platnick *et al.*, 1991). All characters were unweighted. In PAUP, a heuristic search was run utilizing the random stepwise addition sequence and the tree bisection-reconnection branch-swapping algorithm. To insure locating the shortest trees, 100 replicates were run, and a strict consensus was performed on all minimum-length trees. Hennig86 analyses used the "ie*" option to utilize all available tree space, and then a Nelsen consensus tree was calculated for the results. When a family was polymorphic for a character, this was entered as a separate state in Hennig86, as opposed to polymorphic coding for PAUP.

The consensus trees were analyzed with MacClade, version 3.0 (Maddison and Maddison, 1992) for impossible character polarization resulting from the use of "?" for unknown or inapplicable characters in the *Nautilus*/ancestral-coleoid taxon. No such cases were found. Information on character transformation was taken from analyses in MacClade. Data on the ingroup and outgroup were analyzed simultaneously and unrooted, then subsequently rooted between the ingroup and outgroup (Nixon and Carpenter, 1993).

RESULTS

CHARACTER DESCRIPTIONS

Character No. 1: Siphuncle. Character states: 0 - absent; 1- present.

Comments. The presence of a siphuncle is well known in *Sepia, Spirula, Nautilus,* and numerous fossil cephalopods and state 1 (present) is clearly the plesiomorphic condition in coleoids. Less well known is the possible remnant of the siphuncle in *Vampyroteuthis*. In *Vampyroteuthis* a long, very slender duct, continuous with the viscéropericardial (VP) coelom, extends posteriorly from the coelom to an expanded but flattened sac that sits within the apex of the gladius (Fig. 1A). The posterior wall of the sac is complex histologically on its exterior surface but the function is unknown. The pigmented coelomic epithelial lining makes the thread-like duct visible. Pickford (1940) believed this duct to be a remnant of the siphuncle. The siphuncle of living cephalopods contains an extension of the VP coelom and the duct in *Vampyroteuthis* arises in the position where the siphuncle would be expected (*i. e.* body midline). Alternatively the duct could represent the first stage in the reduction of the coelom leading to the octopod condition. In octopods, narrow ducts, found in other locations, resulted from the reduction of the coelom (*i. e.* the "water canals") (see Character 20). In *Vampyroteuthis* the coelom proper terminates well in advance of the conus of the gladius, a condition not found in decapods. Understanding the structure and function of the "end organ" in *Vampyroteuthis* might help resolve the homology of this duct. For the purposes of this study, we have considered the duct to be homologous with the siphuncle.

Character No. 2: Gladius ostracum. Character states: 0 - present; 1- absent.

Comments. The internal shell of many Recent coleoid cephalopods consists of a thin, chitinous, often feather-shaped structure called the gladius (Figs. 2, 3). Bizikov (1991) considered the gladius to consist of three parts: the periostracum (also known as the rostrum); the ostracum (usually the primary component and often the only obvious component of the gladius), and the hypostracum (a cartilage-like thickening of the gladius apparent in some species). Only the ostracum was considered for this character. The shell (cuttlebone) of sepiids looks very different from a gladius. However, if the calcareous material of this shell is dissolved away, a chitinous structure resembling a broad gladius ostracum remains (along with other separable components) (Fig. 2B). We assume this to be the homologue of the gladius. This is not the case in *Spirula* where only components of the septa and siphuncle remain after dissolution of the calcium carbonate. In some other groups (Sepiolidae, Idiosepiidae) the ostracum is reduced in length but this was not considered as a separate character state at the present level of analysis. The cartilage-like shell of the cirrate octopods is superficially, at least, similar to the "hypostracum" of some teuthoids, and we do not consider this or the stylets (shell remnant of some incirrates) to be "ostraca" in the sense employed here.

Character No. 3: Shell composition. Character states: 0 - calcareous material present; 1 - chitin without calcareous material; 2 - without "typical" chitin or calcareous material (*i.e.* cartilaginous); 3- shell absent.

Comments. In most cases the shell composition in this study was estimated just from the visual appearance of the shell. A white, chalky shell as seen in *Nautilus, Sepia,* and *Spirula* was coded as calcareous; a thin, generally amber shell as seen in teuthoids was coded as chitinous; a thick, translucent structure as seen in cirrate octopods was coded as cartilaginous. Octopod stylets were classified as state 2 rather than as a fourth state. Chemical analysis would probably better define these states.

Character No. 4: Internal shell shape. Character states: 0 - flat and elongate; 1 - U-shaped; 2 - coiled; 3 - stylets; 4 -

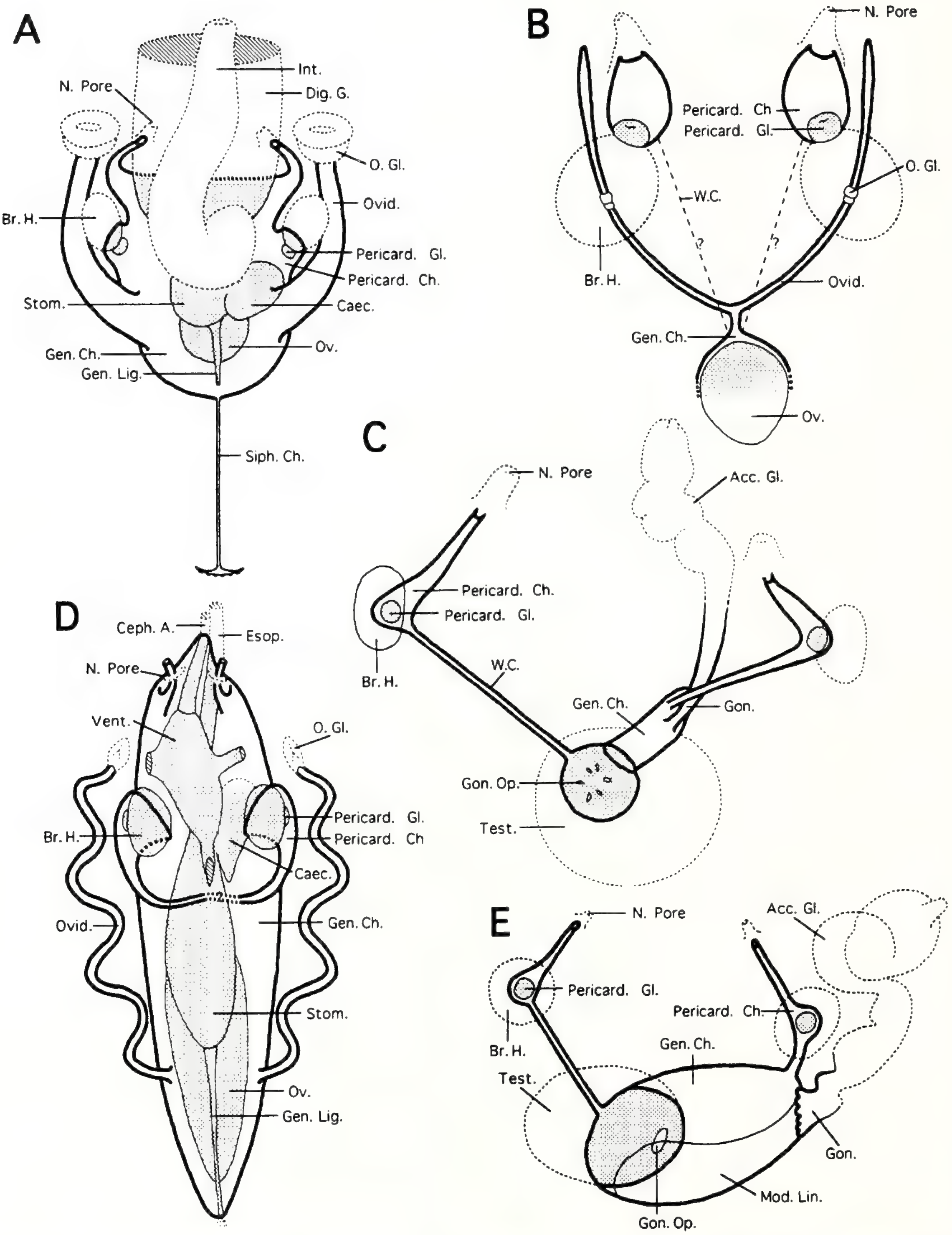
A
Int.
N. Pore
Dig. G.
O. Gl.
Ovid.
Br. H.
Pericard. Gl.
Pericard. Ch.
Stom.
Caec.
Gen. Ch.
Ov.
Gen. Lig.
Siph. Ch.
B
N. Pore
Pericard. Ch
Pericard. Gl.
O. Gl.
W.C.
?
?
Br. H.
Ovid.
Gen. Ch.
Ov.
C
N. Pore
Acc. Gl.
Pericard. Ch.
Pericard. Gl.
Br. H.
W.C.
Gen. Ch.
Gon.
Gon. Op.
Test.
D
Ceph. A.
Esop.
N. Pore
Vent.
O. Gl.
Pericard. Gl.
Pericard. Ch
Br. H.
Caec.
Ovid.
Gen. Ch.
Stom.
Ov.
Gen. Lig.
E
N. Pore
Acc. Gl.
Pericard. Gl.
Pericard. Ch.
Br. H.
Gen. Ch.
Test.
Gon.
Mod. Lin.
Gon. Op.

Fig. 1. Visceropericardial coelom. A. *Vampyroteuthis*. B. *Japetella*. C. *Grimpoteuthis*. D. *Sthenoteuthis*, shaded portions of the ink sac, branchial hearts, and intestine are covered by coelomic lining (*i.e.* they "lie" within the coelomic cavity). E. *Stauroteuthis*. (Acc. Gl., accessory gland; Br. H., branchial heart; Caec., caecum; Ceph. A., cephalic artery; Dig. G., digestive gland; Esop., esophagus; Gen. Ch., genital chamber of coelom; Gen. Lig., genital ligament; Gon., gonoduct; Gon. Op., gonadal opening to coelom; Int., intestine; Mod. Lin., modified lining; N. Pore, nephridial pore; O. Gl., oviducal gland; Ov., ovary; Ovid., oviduct; Pericar. Ch., pericardial chamber of coelom; Pericar. Gl., pericardial gland; Siph. Ch., siphuncular chamber of coelom; Stom., stomach; Test., testis; Vent., ventricle; W.C., water canal, thickness exaggerated).

NA (absent).
Comments. This character refers to the general shape of the internal shell. "Elongate" refers to elongation in the direction of the body axis (Fig. 3A). Stylets are elongate but not strictly in the direction of the body axis (Fig. 10B) and are not flattened. NA (not applicable) refers to the loss of an internal shell in some incirrate octopods. Under "U-shaped" we included the various modifications found in cirrate octopods (see Voss, 1988).

Character No. 5: Buccal crown. Character states: 0 - absent; 1 - present as oral arms; 2 - present.
Comments. The buccal crown consists of the muscular buccal supports and the connecting membrane that surrounds the mouth and lips (Fig. 4A). Naef (1928) considered the oral arms (inner ring of tentacles) of *Nautilus* (Fig. 4B) to be homologous with the buccal crown of decapods because of their similar location and because the buccal supports in decapods arise from secondary budding off the arm buds (in Boletzky, 1993b). Also, the buccal supports resemble arms in their possession of suckers in a few families (Fig. 4A). We, therefore, consider the presence of a buccal crown to be plesiomorphic within the Coleoidea.

Character No. 6: Arms II. Character states: 0 - unmodified; 1- filaments; 2 - absent.
Comments. The presence of ten equal arms in early coleoid fossils (*e. g. Jeletzkya*, Middle Pennsylvanian) suggests that the ancestral coleoid had ten equal arms. Naef (1928) noted that in the developing octopod embryo the primary folds from which the eyelids derive lie between arms II and III while in the decapods they lie between arms III and IV suggesting that the missing arm in octopods was one of the first three pairs (in Boletzky, 1993a). Because the first arm rudiment is widely separated from the others only in decapods, he suggested that the first arms were missing in octopods. Boletzky (1978-1979), however, found another marker, the metabrachial vesicles, that lies between arms I and II in both octopods and decapods and suggested that the missing arms are either arms II or III. The filaments in *Vampyroteuthis* lie in the position of a second pair of arms (Fig. 3A). R. E. Young (1967) found that the primary nerve trunks between the filaments and the subesophageal lobes of the brain largely bypassed the brachial lobe and suggested that the filaments might be homologous with the pre-ocular tentacles of *Nautilus*. On the other hand, J. Z. Young (1977) found, by dissection, nerves extending from the filament to each of two ganglia

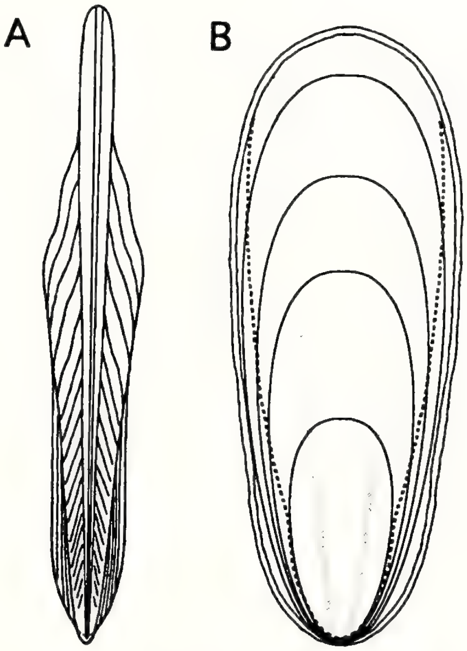

Fig. 2. Gladius ostracum. A. *Abralia*. B. *Sepia*, extracted from cuttlebone by dissolving calcium carbonate in weak acid. Ovals are representative growth lines; oblique lines mark inner margins of thickened layers on the ventral surface of the ostracum.

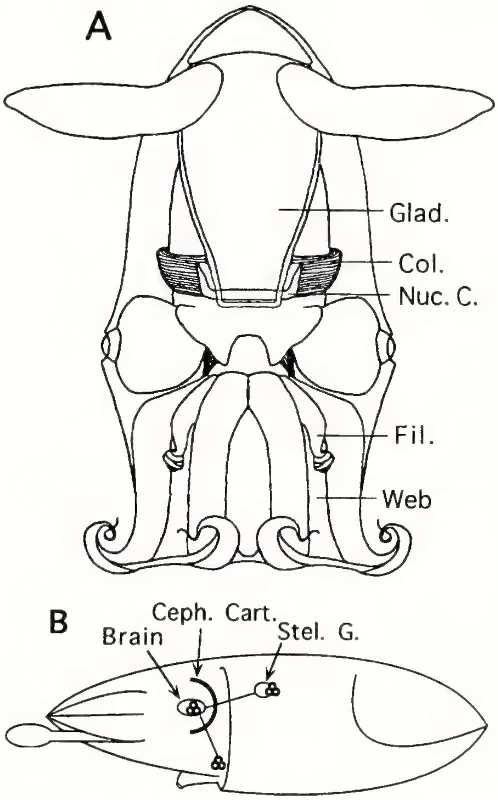

Fig. 3. A. Diagrammatic illustration of *Vampyroteuthis* showing position of the filaments, nuchal cartilage and gladius (modified after R. E. Young, 1962). B. Generalized cephalopod showing positions occupied by photosensitive vesicles. (Ceph. Cart., cephalic cartilage; Col., collar muscle; Fil., filament; Glad., gladius; Nuc. C., nuchal cartilage; Stel. G., stellate ganglion).

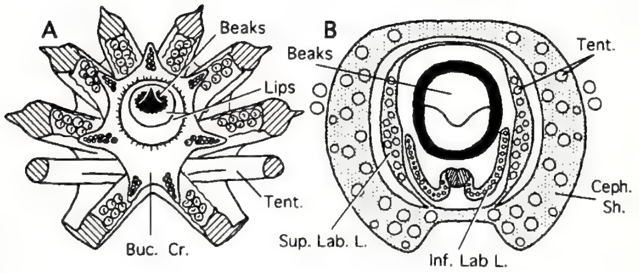

Fig. 4. Oral view of arm crowns (diagrammatic). A. *Loligo* (after Naef, 1921-1923) showing armature of arms in two series and suckers on the buccal crown. B. *Nautilus* (modified after Griffin, 1900). (Buc. Cr., buccal crown; Ceph. Sh., cephalic sheath; Inf. Lab. L., inferior labial lobe bearing tentacles; Sup. Lab. L., superior labial lobe bearing tentacles; Tent., tentacle).

on the circumoral commissure (= nerve ring). The latter connects the axial nerves of all the arms. He considered this proof that the filaments were modified arms II. We have made several attempts to confirm the existence of these connecting nerves to the filaments but failed to find them. Numerous small nerves radiate from the ganglia on the circumoral commissure to various muscles. In addition, the region is crossed by numerous slender muscle or connective tissue fibers. As a result, the nervous connections could be easily misinterpreted. The possible absence of a connecting nerve again opens the question of the homology of the filaments but does not disprove their origin from the second pair of arms. Indeed, the axial nerve of the tentacles (fourth arms) of some decapods (*e. g. Loligo, Sepia*) does not have a connection with the circumoral ring (Fig. 5B).

For this study, character coding was based on the assumptions that (1) the octopods have lost arms II, and that (2) the vampire filaments are modified arms II. Under these assumptions, the character states can be analyzed as "ordered": unmodified - filaments - absent.

Character No. 7: Arms IV. Character states: 0 - unmodified; 1 - tentacles.
Comments. Decapods retain the ten arms of the ancestral coleoid but arms IV have been modified into tentacles (Fig. 4A).

Character No. 8: Sucker rings. Character states: 0 - cuticular rings; 1 - no rings; 2 - horny rings.
Comments. This character refers to the secreted linings of suckers. The cuticular lining in octopods is chitinous (Hunt

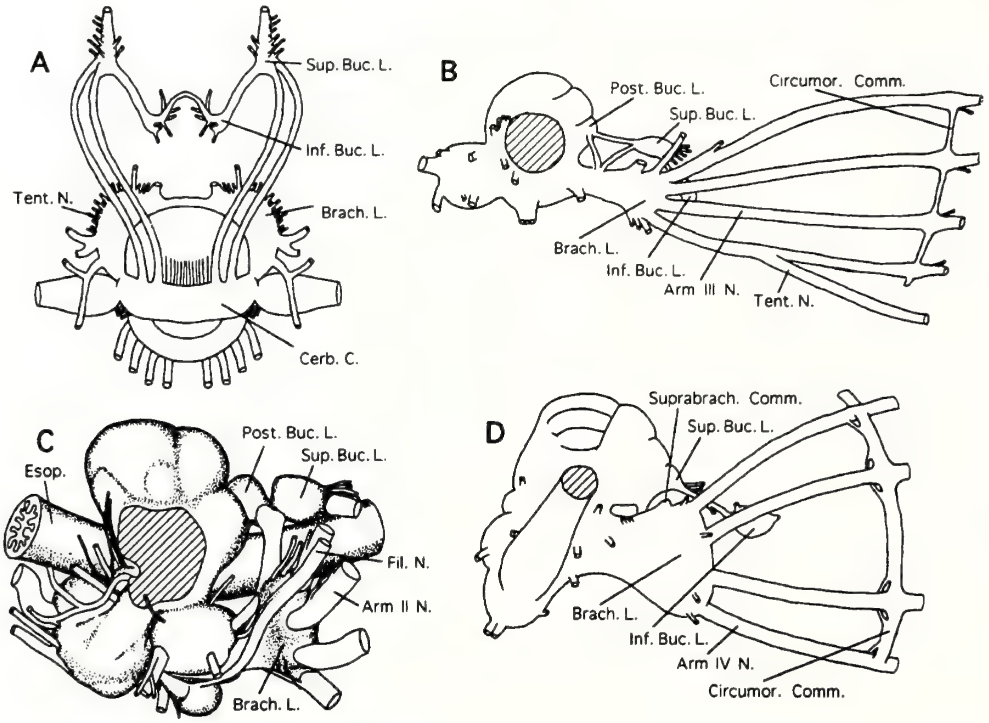

Fig. 5. Central nervous system (brain). A. *Nautilus* (modified after Griffin, 1900). B. *Sepia* (modified after Hillig, 1913). C. *Vampyroteuthis* (modified after R. E. Young, 1967). D. *Octopus* (modified after J. Z. Young, 1971). Arm numbering between taxa is based on morphological position not necessarily homology. (Arm II N., arm II axial nerve; Arm III N., arm III axial nerve; Arm IV N., arm IV axial nerve; Brach. L., brachial lobe; Cerb. C., cerebral cord; Circumor. Comm., circumoral commissure; Esop., esophagus; Fil. N., filament axial nerve; Inf. Buc. L., inferior buccal lobe; Post. Buc. L., posterior buccal lobe; Sup. Buc. L., superior buccal lobe; Suprabrach. Comm., suprabrachial commissure; Tent. N., tentacle nerve).

and Nixon, 1981). The inner "chitinous" sucker rings found in decapods are thick, generally bear teeth, and are more properly termed "horny" rings because they do not contain chitin (at least in *Sepia*; Rudall, 1955). Otherwise, the chemical composition of the decapod rings is unknown (Nixon and Dilly, 1977). Nixon found no trace of a cuticular lining in *Vampyroteuthis* and our sections confirm her observations. Neither we nor Nixon, however, had suckers in perfect condition.

Character No. 9: Sucker stalks. Character states: 0 - base and neck; 1 - base and plug; 2 - cylinder.
Comments. In octopods, a sucker stalk consists of a broad cylinder of muscles that attaches to the outer lateral walls of the acetabulum often at the point where the latter joins the infundibulum (Fig. 6A). Also, oblique muscle fibers cross within the cylinder (Graziadei, 1971). In decapods, a sucker stalk consists of a broad conical base that tapers gradually or abruptly to a neck of varying length (Fig. 6B). The neck is a narrow muscular rod that attaches off-center within the base of the sucker acetabulum. Superficially the sucker stalks in *Vampyroteuthis* resemble those of the decapods in having a broad base and a short neck (Fig. 6C). Both components, however, differ substantially from the decapod condition. The neck attaches (virtually adhears) to the connective tissue at the base of the acetabulum. In decapods the neck muscles and other tissues invade and form part of the acetabulum. The base in *Vampryoteuthis* does not clearly attach as a unit to the arm muscles. The major attachment is a band of muscles that runs proximally from the sucker neck, along the midline of the arm, to attach to the arm muscles beneath the base of the preceeding sucker. The relationships of the peculiar sucker stalk of *Vampyroteuthis* to that of the decapods and octopods is not clear at present and we have considered it a separate character state.

Character No. 10: Sucker symmetry. Character states: 0 - radial symmetry; 1 - bilateral symmetry.
Comments. The sucker is radially symmetrical in *Vampyroteuthis* and the octopods but strongly bilateral in the decapods (Fig. 6). The bilaterality of the latter is most apparent in the shape of the horny rings and the point of attachment to the stalk.

Character No. 11: Arm III armature series. Character states: 0 - one; 1 - two; 2 - four; 3 - more than four.
Comments. "Armature series" refers to the number of sucker and/or hook series paralleling the arm axis in the midportion of the arm (Fig. 4A). We restricted the character to the arm midportion as the armature series often differs at the tips and bases of the arms. In incirrate octopods, suckers almost invariably begin in embryos as a single series regardless of the number of series in the adults (Naef, 1921-1923). This suggests that a single series is plesiomorphic in the Octopoda. In decapods, hatchlings of many species have two or more rows and the plesiomorphic state is uncertain. *Nautilus* has a series of rings encircling the arms that can form a suction on their oral surfaces. This could be interpreted as a precursor to a single sucker series in early coleoids. This possibility, however, is contradicted by the presence of two rows of hooks in one of the oldest coleoids (*Jeletzkya*).

Character No. 12: Arm V sucker series. Character states: 0 - one; 1- two; 2 - four; 3 - more than four.
Comments. Armature series on arms V is not always the same as on arms III. As a result we regarded this as a separate character. As above the armature series refers to that in midarm.

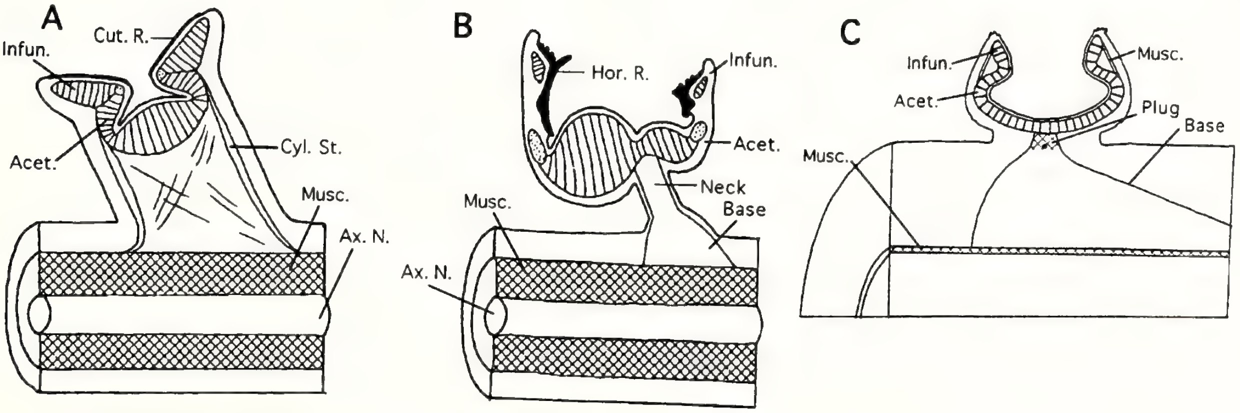

Fig. 6. Longitudinal sections of suckers (diagrammatic). A. *Octopus* (modified after Naef, 1921-1923). B. *Loligo* (modified after Naef, 1921-1923). C. *Vampyroteuthis*. (Acet., acetabulum; Ax. N., axial nerve; Cut. R., cuticular ring; Cyl. St., cylindrical stalk; Hor. R., horny ring; Infun., infundibulum; Musc., muscle).

Character No. 13: Trabeculae on unmodified arms. Character states: 0 - present; 1 - absent.
Comments. "Unmodified" refers to arms not modified for sexual functions. Trabeculae, in their most recognizable form, are conical, muscular structures that arise lateral to the suckers on the arms and are attached to the muscular cylinder of an arm. In some cases the identification of trabeculae is difficult without sectioning. Our character states were coded on the basis of general appearance and not histological sections. We, therefore, defined trabeculae as "present" only when they were clearly recognizable from their associated membranes. Therefore, if trabeculae are present but obscure, they will be incorrectly coded as absent. When membranes are lacking, possible trabeculae can be confused with skin folds associated with sucker bases. In this case, a distinct muscular pillar must be recognized to qualify as a trabecula. Trabeculae are not to be confused with the muscular sucker bases with which they are often associated. The origin of trabeculae is uncertain but they are similar in structure to sucker bases and, indeed, in a variety of cephalopods sucker bases are modified into structures apparently identical with trabeculae (*e. g.* on hectocotylized arms in a variety of squids, on the distal tips of the arms in *Vampyroteuthis*). Although the trabeculae are modified in a variety of ways in decapods, in many (*e. g. Thysanoteuthis*) the basic structure and attachment are identical to the cirri of octopods. Naef (1921-1923) considered trabeculae and cirri to be homologues and they were treated so in this study.

Character No. 14: Protective membranes on unmodified arms. Character states: 0 - absent; 1 - present.
Comments. "Unmodified" refers to arms not modified for sexual functions. An arm protective membrane is a membrane that connects trabeculae and lies lateral to the suckers/hooks. When trabeculae cannot be clearly recognized, the definition changes to: a membrane that is uninterrupted, at least along its free margin, by sucker bases. The octopod, *Ocythoe*, for example has membranes that are completely interrupted by suckers (*i. e.* the membrane extends from sucker to sucker) and, therefore do not qualify as "protective membranes" as defined here. To code this character as present, membranes need be present only on a portion of an arm, *e. g. Vampyroteuthis* which has protective membranes only near the arm tips (Pickford, 1946). There is no relevant character state for *Nautilus.*

Character No. 15: Well-developed interbrachial web between arms I. Character states: 0 - virtually absent; 1 - present.
Comments. "Well-developed" means the center of the web extends more than 20% of the arm length measured from the point where adjacent arms join (Fig. 3A). The sector between arms I was picked because this sector often has the lowest web development among the dorsal arms. Coding of this character was somewhat subjective in a few cases.

Character No. 16: Well-developed interbrachial web between arms V. Character states: 0 - absent; 1 - present.
Comments. "Well-developed" means the center of the web extends more than 20% of the arm length. The sector between arms V was picked because this sector generally has the lowest web development among the ventral arms. The absence of a web between arms V is often independent of the web condition of the dorsal arms. *Nautilus* lacks a relevant character state.

Character No. 17: Fin cartilage. Character states: 0 - at proximal end of fin; 2 - at proximal end and in core; 3 - NA (fins absent).
Comments. In decapods the fins typically insert on a flattened cartilage with a slight medial ridge; the cartilage attaches to the shell sac. In cirrates the fin cartilage has generally been considered to be absent (Robson, 1932). In *Vampyroteuthis* the problem of comparison is complicated by the fact that this animal has two pairs of fins. The juvenile fin appears first and the adult fin later in a more anterior position; with growth the juvenile fins are resorbed and the adult fins enlarge (Pickford, 1950). In dissecting and sectioning the fins of cirrates, we found that they have a fin cartilage with a small "base" that adheres to the shell sac and an extensive "plate" that occupies the core of the proximal half of the fin (Figs. 7A-D). The cartilage consists of a highly vacuolated tissue with virtually no matrix other than the thin walls of the vacuoles. The flexible, spongy consistency is apparently responsible for it not being previously recognized as cartilage. The adult vampire fin has a small cartilage at the tip of the attached end of the fin and the fin has an L-shape that is very different from those of cirrates or decapods (Figs. 7E, F). The juvenile fin of *Vampyroteuthis* has an internal cartilage similar to that of the cirrates but with a much larger flattened, cartilaginous "base" attached to the shell sac and a slender cartilaginous "plate" (continuous with the base) that extends through the entire core of the fin (Figs. 7G, H). The histology is the same as in the cirrates except that the vesicles are larger in *Vampyroteuthis* (Fig. 7I). We consider the juvenile fin of *Vampyroteuthis* to be the homologue of the octopod and decapod fins.

The absence of fins in incirrates is clearly secondary as their anlagen are present in the embryo but disappear during development (Naef, 1921-1923).

Character No. 18: Statocyst outer capsule. Character states: 0 - outer capsule absent; 1 - outer capsule present.
Comments. Statocysts have one of two basic structures in

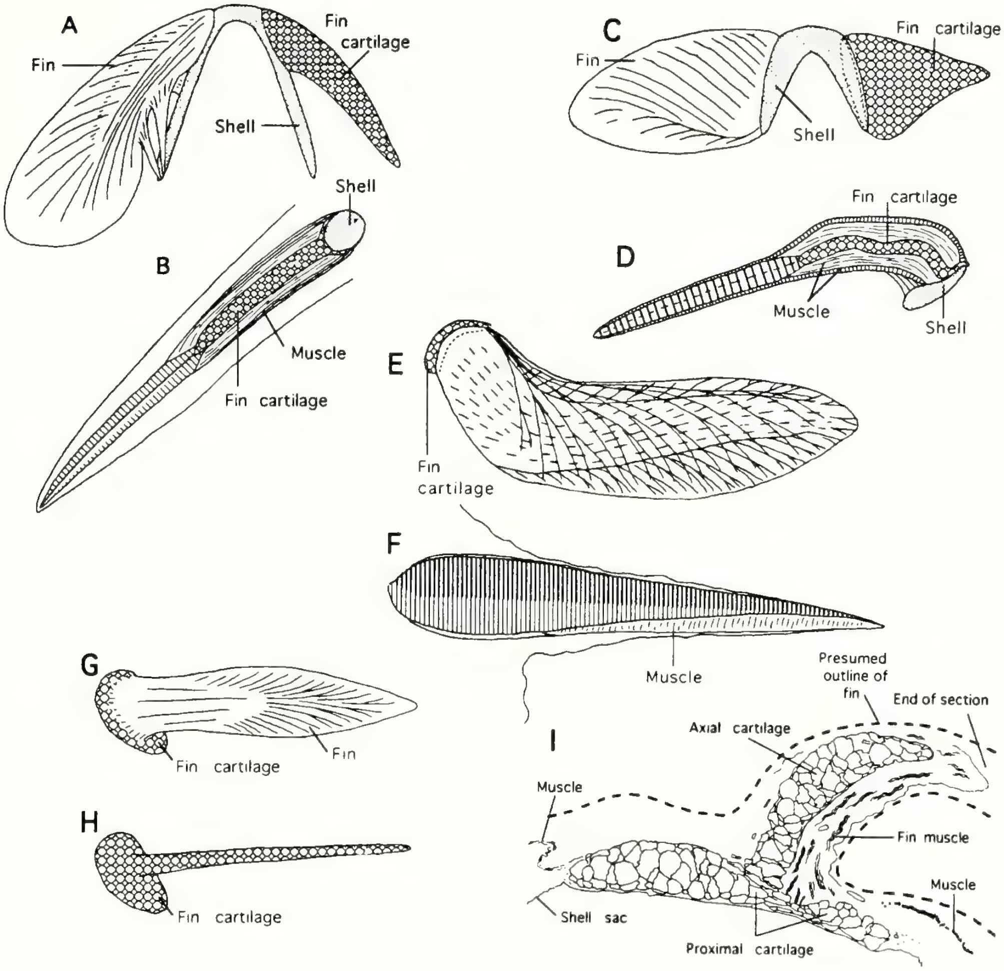
A
Fin
Fin cartilage
Shell
C
Fin
Fin cartilage
Shell
Shell
B
Muscle
Fin cartilage
Fin cartilage
D
Muscle
Shell
E
Fin cartilage
F
Muscle
Presumed outline of fin
End of section
G
Fin cartilage
Fin
I
Axial cartilage
Muscle
Fin muscle
Muscle
Shell sac
Proximal cartilage
H
Fin cartilage

Fig. 7. Fins (diagrammatic). A. *Grimpoteuthis*, ventral view with outer tissue removed. B. *Grimpoteuthis*, longitudinal section through fin. C. *Stauroteuthis*, ventral view with outer tissue removed. D. *Stauroteuthis*, longitudial section through fin. E. *Vampyroteuthis*, adult fin, dorsal view. F. *Vampyroteuthis*, adult fin, longitudinal section through straight portion of fin. G. *Vampyroteuthis*, juvenile (= "larval") fin, dorsal view. H. *Vampyroteuthis*, juvenile fin, dorsal view with outer tissue removed. I. *Vampyroteuthis*, longitudinal section through juvenile fin.

cephalopods: they consist of a single sac that is commonly embedded directly in cartilage or they lie within an additional fluid-filled sac, the outer capsule, which is commonly embedded in cartilage.

Character No. 19: Nephridial coelom. Character states: 0 - nephridial coeloms separate (unipaired); 1 - nephridial coeloms fused (single coelom).
Comments. In *Vampyroteuthis infernalis*, left and right nephridial sacs are separated from one another by their medial walls (Fig. 8A) and further by the intestine which lies between the medial walls at their ventral ends (Fig. 8B). Each has a rather simple shape and includes renal appendages arising from (1) the cephalic vein, (2) vena cava en route to the branchial heart, and (3) a dorsal branch of the vena cava (the latter forms an abbreviated dorsal lobe).

In the incirrate *Japetella diaphana* the left and right nephridial sacs are, also, entirely separate and a more distinct dorsal lobe is present (Figs. 8C, D).

The nephridial coelom has not been well described in the teuthoids. In dissecting *Sthenoteuthis oualaniensis* (Lesson, 1830) we found the coelom divided into two chambers: the dorsal and ventral sacs (Figs. 8E, F). The ventral sac is roughly Y-shaped with the stem of the Y directed anteriorly and the arms defined by a septum that partially divides the cavity posteriorly. The cephalic vein enters the cavity dorsally. The renal appendages are restricted to the ventral sac although the cephalic vein has what seems to be tiny appendages near its dorsal entrance. The ventral sacs surround the intestine, ink sac, and the extensive lobes of the digestive gland duct appendages (DGDA) (see Character 43). Communication between the dorsal and ventral sacs occurs to either side of the inflated

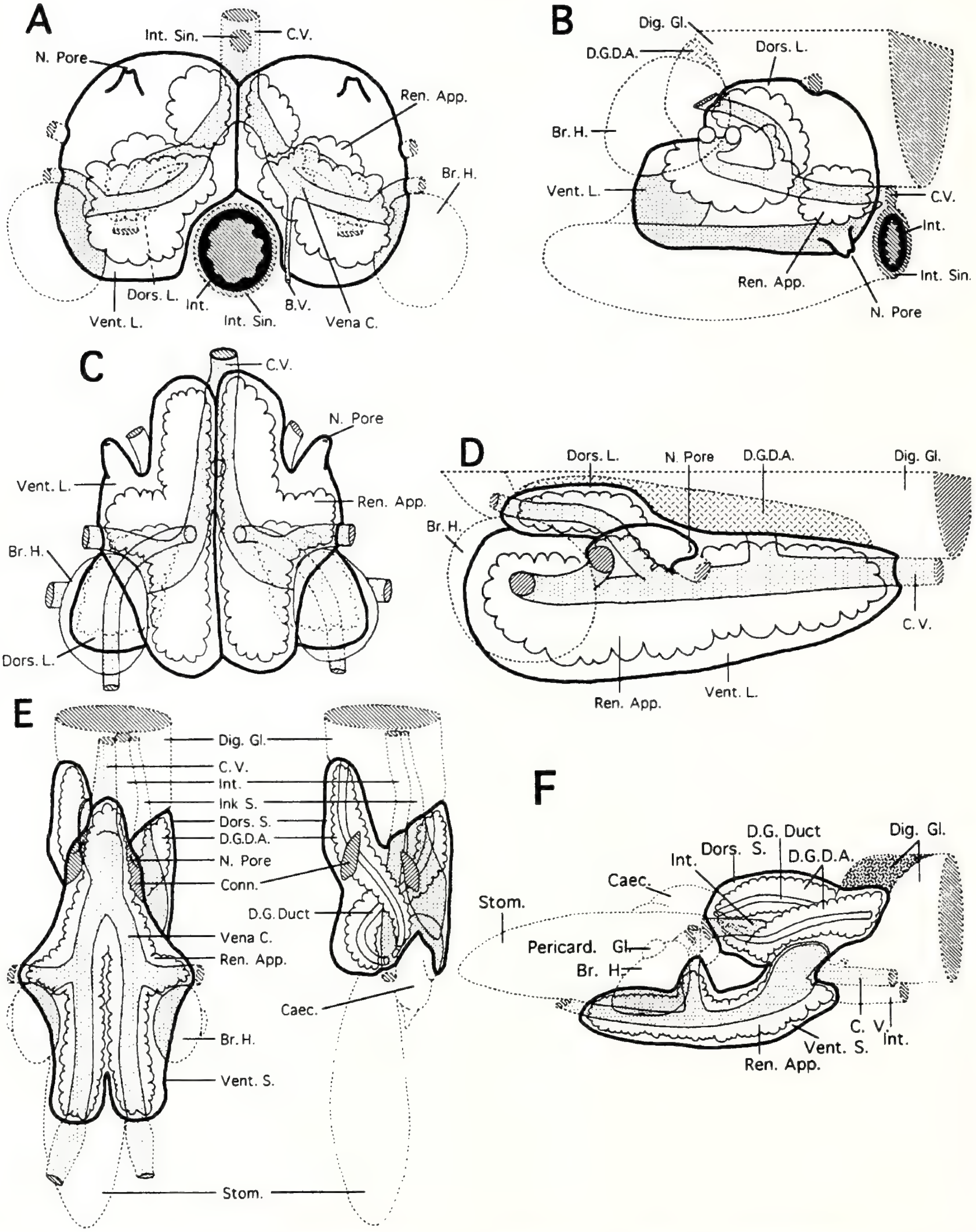
A
Int. Sin.
C.V.
N. Pore
Ren. App.
Br. H.
Dors. L.
Int.
B.V.
Vent. L.
Int. Sin.
Vena C.
B
Dig. Gl.
D.G.D.A.
Dors. L.
Br. H.
Vent. L.
C.V.
Int.
Int. Sin.
Ren. App.
N. Pore
C
C.V.
N. Pore
Vent. L.
Ren. App.
Br. H.
Dors. L.
D
Dors. L.
N. Pore
D.G.D.A.
Dig. Gl.
Br. H.
C.V.
Ren. App.
Vent. L.
E
Dig. Gl.
C.V.
Int.
Ink S.
Dors. S.
D.G.D.A.
N. Pore
Conn.
D.G. Duct
Vena C.
Ren. App.
Caec.
Br. H.
Vent. S.
Stom.
F
D.G. Duct
Dig. Gl.
Dors. S.
Int.
D.G.D.A.
Caec.
Stom.
Pericard. Gl.
Br. H.
C. V.
Int.
Vent. S.
Ren. App.

Fig. 8. Nephridial coelom (diagrammatic). A. *Vampyroteuthis*, ventral view. B. *Vampyroteuthis*, side view. C. *Japetella*, ventral view. D. *Japetella*, side view. E. *Sthenoteuthis*, left: ventral view; right: ventral view with ventral sac removed. F. *Sthenoteuthis*, side view. (B. V., blood vessel; Br. H., branchial heart; Caec., caecum; Conn., connection between dorsal and ventral sacs of coelom; C. V., cephalic vein; D. G. D. A., digestive gland duct appendages; D. G.Duct, digestive gland duct; Dig. G., digestive gland; Dors. L., dorsal lobe of coelom; Dors. S., dorsal sac of coelom; Ink S., ink sac; Int., intestine; Int. Sin., intestinal sinus; N. Pore, nephridial pore; Pericard. G., pericardial gland; Ren. App., renal appendages; Stom., stomach; Vena C., vena cava; Vent. L., ventral lobe of coelom; Vent. S., ventral sac of coelom).

cephalic vein as it enters the ventral sac. This is generally the same as in *Sepia* (Tompsett, 1939).

In the cirrate *Grimpoteuthis glacialis* (Robson, 1930) the nephridial sacs are also paired (Fig. 9A). Each sac has a very extensive dorsal lobe. The right sac has a dorsal branch that extends posteriorly as a large sac that circles the gonad to its posterior tip and contains renal appendages throughout most of its course. The dorsal branch of the left sac also has a posterior extension but it is very broad and lacks renal appendages although they are present dorsally.

In the cirrate *Stauroteuthis syrtensis* the nephridial sacs are paired as well (Fig. 9B). The dorsal branch off the vena cava is longer than in *Japetella* and carries the bulk of the renal appendages. The dorsal lobe, therefore, is large but its full extent was not mapped.

This character can be difficult to evaluate in preserved specimens. In general if the renal appendages had a Y-shaped morphology along their ventral face (*i. e.* renal appendages begin on the cephalic vein), we assumed that the coelom of either side was continuous (fused) medially.

Character No. 20: Visceropericardial coelom. Character states: 0 - extensive coelom (surrounds visceral nucleus, ventricle and gonad); 1 - coelom reduced (viscera excluded except part of the gonad).
Comments. We have illustrated the visceropericardial (VP) coelom of several species which have not been illustrated previously. The VP coelom of *Vampyroteuthis* (Fig. 1A) surrounds most of the visceral nucleus, much of the posterior end of the digestive gland including part of the digestive gland duct appendages (but see Character 43), posterior end of the crop, ventricle, and the gonad which is suspended in the coelom from the genital strand. The pericardial chamber is represented by short outpocketings on either side of the coelom that enclose the pericardial glands and the medial portions of each branchial heart. At its antero-lateral corners the coelom narrows into ducts that open into the nephridial coelom at the base of the nephridial papillae.

In *Japetella diaphana* the VP coelom (Fig. 1B) is very restricted in extent as appears typical of the incirrate octopods (see Isgrove, 1909). We were, however, unable to locate the water canals but they could have been missed. The degree to which the gonad lies in the coelom could not be fully evaluated as the tissue layers did not separate cleanly. However, the ventral half of the gonad and part of the anterior portion appeared to be within the coelomic cavity.

In *Grimpoteuthis glacialis* the VP coelom is very restricted but less so than in *Japetella* (Fig. 1C). It covers just a small patch of the gonad (here the gonad opens into the coelom). This portion is separated from the rest of the coelomic sac (the outer sac) by a transverse membrane that partially occludes the connection. The gonoduct and the left water canal open into the outer sac near its lateral extent and the right water canal opens into the inner sac. Both water canals extend to a small sac covering the pericardial gland. Anterior ducts from the latter sacs open into the nephridial coelom at the base of the nephridial papillae. There appears to be no genital pocket in the male.

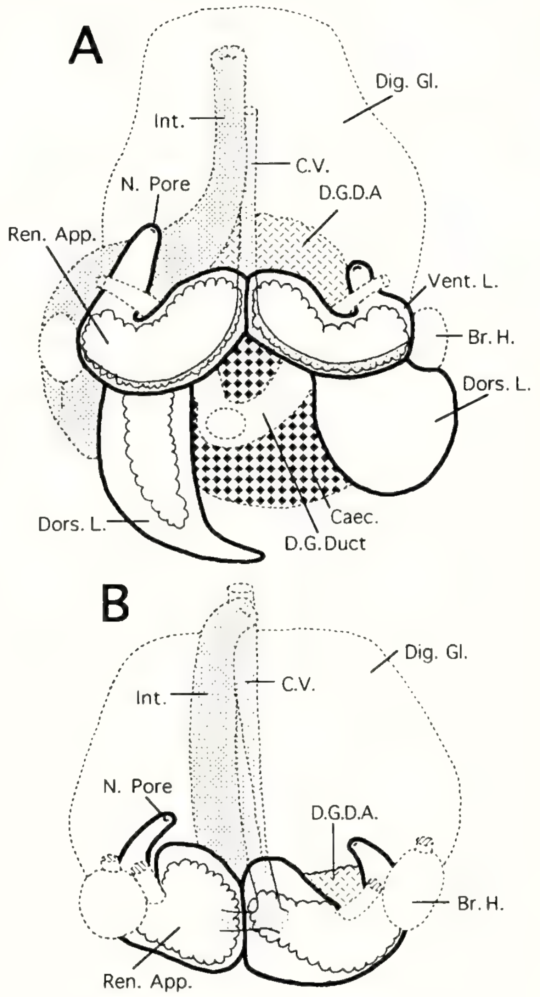

Fig. 9. Nephridial coelom (ventral view, diagrammatic). A. *Grimpoteuthis*. B. *Stauroteuthis*. (Br. H., branchial heart; C. V., cephalic vein; Caec., caecum; D. G. D. A., digestive gland duct appendages; D. G. Duct, digestive gland duct; Dig. G., digestive gland; Dors. L., dorsal lobe of coelom; Int., intestine; N. Pore, nephridial pore; Ren. App., renal appendages; Vent. L., ventral lobe of coelom).

The VP coelom of *Sthenoteuthis oualaniensis* is generally representative of the decapods (Fig. 1D). It extends from the digestive gland to the conus of the gladius and incorporates most of the visceral nucleus (stomach and caecum), ventricle, posterior esophagus, and the gonad. The pericardial chamber of the VP coelom consists of a shelf on the ventral surface of the coelom and encloses much of the branchial hearts and pericardial glands and appears to be continuous across the ventral midline although we could not be certain of this. Anteriorly the VP coelom narrows abruptly into ducts which extend through the nephridial pores to open into the mantle cavity. The extension of the ducts into the mantle cavity could be a peculiarity of this species.

In *Stauroteuthis syrtensis*, the VP coelom is less restricted than in *Grimpoteuthis* and is reduced mostly to a sac that covers, at its right end, a portion of the gonad and leads, at its left end, into the large fluted end of the broad male gonoduct (Fig. 1E). Two narrow ducts ("water canals") extend from the coelomic sac to include the pericardial glands. The right duct is long and slender and the left one short and somewhat broader (the thickness of both is exaggerated in the illustration). From the sac around the pericardial gland a broader and more muscular duct opens into the nephridial coelom at the base of the nephridial papilla on either side. There appears to be no genital pocket in the male.

In *Nautilus*, the VP coelom is extensive and divided into pericardial, genital, and siphuncular chambers. The gonad, part of the intestine, stomach, digestive gland, and ventricle are covered by coelomic epithelium (Griffin, 1900).

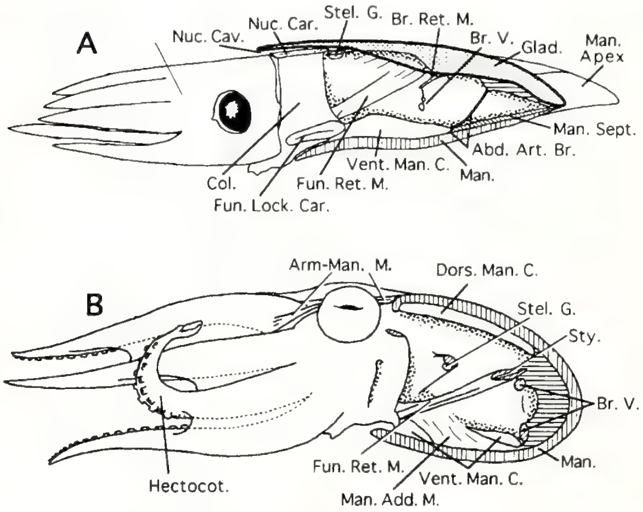

Fig. 10. The teuthoid *Abralia* showing the mantle septum, nuchal cartilage, nuchal cavity, funnel-locking cartilage, and position of stellate ganglion. Heavy line outlines gladius. B. An incirrate octopodid showing the dorsal mantle cavity, position of stellate ganglion, muscle connection between arm bases and anterior mantle margin, mantle adductor muscle, stylets, and hectocotylus. [Abd. Art. Br., abdominal (median pallial) artery and branch (lateral pallial artery); Arm-Man. M., arm-mantle muscle; Br. Ret. M., branchial retractor muscle; Br. V., branchial blood vessel; Col., collar; Dors. Man. C., dorsal mantle cavity; Fun. Lock. Car., funnel locking cartilage; Fun. Ret. M., funnel retractor muscle; Glad., gladius; Hectocot., hectocotylus; Man., mantle; Man. Add. M., mantle adductor muscle; Man. Apex, mantle apex; Man. Sept., mantle septum; Nuc. Car., nuchal cartilage; Nuc. Cav., nuchal cavity; Stel. G., stellate ganglion; Sty., stylet; Vent. Man. C., ventral mantle cavity].

Character No. 21: Dorsal mantle cavity. Character states: 0 - absent; 1 - present.
Comments. This cavity is a dorsal continuation of the ventral mantle cavity, across the dorsal midline, posterior to the stellate ganglia (Fig. 10B). The large dorsal cavity of *Spirula* is excluded from state 1 by its presence anterior to the stellate ganglia and we consider it to be a "nuchal" cavity. Because *Nautilus* lacks stellate ganglia, we define the dorsal mantle cavity, in this case, as a cavity well posterior to the level of the collar. The cavity in the collar region, the "nuchal" cavity, is characteristic of most cephalopods in which the mantle and head are not fused (Fig. 10A). The dorsal mantle cavity as defined here proved to be characteristic of the octopods.

Character No. 22: Nidamental glands. Character states: 0 - absent; 1 - present.
Comments. Nidamental glands of decapods are large, paired organs that open directly into the mantle cavity and are composed of numerous lamellae that are involved in secretion of egg cases or masses. *Nautilus* has a three-lobed, lamellar nidamental gland.

Character No. 23: Crop. Character states: 0 - present; 1 - absent.
Comments. The crop is an expansion or a diverticulum of the esophagus for food storage. For this study, we consider a crop to be absent unless it is morphologically obvious. The cirrate octopods we examined have an esophagus that is only slightly expanded and we considered this as state 1. Others (see Robson, 1932) have indicated the presence of a reduced crop in cirrates which could simply reflect differences in how a crop is defined. These statements, nevertheless, have resulted in our coding the cirrates as polymorphic for this character.

Character No. 24: Branchial canal. Character states: 0 - absent; 1 - present.
Comments. The branchial canal is a large opening at the base of each gill lamella and between the primary afferent and efferent blood vessels of the gill (Figs. 11A, D, E). This canal allows passage of sea water between lamellae at this point. This unambiguous character was a primary feature used by systematists for many years to separate teuthoids from sepioids.

Character No. 25: Mantle septum. Character states: 0 - absent; 1 - present and continuous; 2 - present but open posteriorly; 3 - blood vessel only.

Comments. The mantle septum passes from the ventral surface of the visceral mass, across the mantle cavity to the inner surface of the mantle wall (Fig. 10A). The membrane lies in an anterior/posterior orientation and divides the mantle cavity into right and left sides. Along its anterior margin, the membrane supports, in decapods, a branch of the abdominal aorta as it passes from the visceral mass to the ventral mantle wall. Character state 3 occurs only in *Spirula* where the coiled shell leaves room for only the artery. No septum is present in *Nautilus*; however, the very different organization of the mantle cavity suggests that this character is not applicable to *Nautilus.*

Character No. 26: Mantle adductor. Character states: 0 - absent; 1 - present; 2 - NA.
Comments. The mantle septum commonly has slender muscle fibers running along it primarily in an anterior-posterior direction. In some cephalopods, a pronounced muscle bundle, the mantle adductor, is present that runs more or less ventrally from the visceral mass to the mantle wall (Fig. 10B). Presumably an adductor cannot exist without a mantle septum as a precursor. NA refers to the absence of the mantle septum. *Nautilus* lacks a relevant character state.

Character No. 27: Funnel valve. Character states: 0 - present; 1 - absent.
Comments. The funnel valve is a muscular flap, continuous with the postero-dorsal wall of the funnel, that lies within the lumen of the funnel.

Character No. 28: Nuchal cartilage. Character states: 0 - present; 1 - absent.
Comments. The nuchal cartilage is the support for the head component of the nuchal locking apparatus (Fig. 10A). Muscles of the collar and muscles from the head and shell sac attach to this cartilage. In *Vampyroteuthis*, although the head and mantle are fused, a nuchal cartilage remains (Fig. 3A). Its shape varies from a flat rectangle to a low U-shape and lies beneath and just posterior to the anterior end of the cartilage that surrounds much of the periphery of the gladius. The cartilage no longer supports a locking apparatus but still provides a site for muscle attachment. *Nautilus* lacks a mantle-propulsion system which, presumably, is a prerequisite for development of a nuchal cartilage; it has no relevant character state.

Character No. 29: Cornea. Character states: 0 - cornea absent; 1 - one-part cornea present; 2 - two part cornea present.
Comments. The cornea is a transparent, protective covering of the lens. Here we consider all one-part corneas as homologues; although this is a debatable assumption it is not critical at this level of analysis. *Nautilus* lacks a relevant character state because its eyes do not bear lenses.

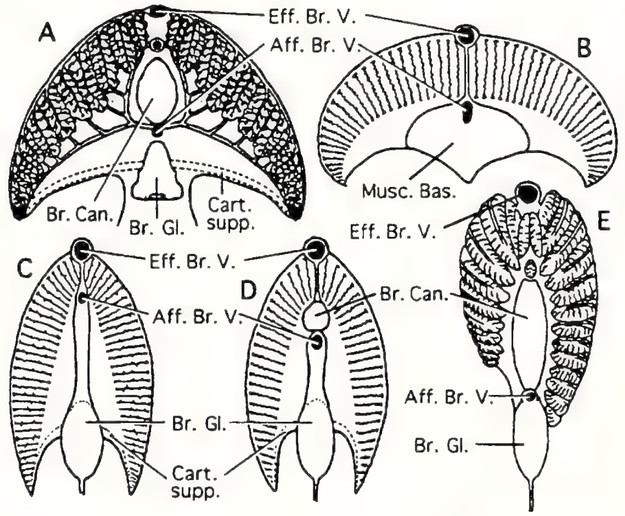

Fig. 11. Gill filaments (diagramatic). A. *Vampyroteuthis* (modified after R. E. Young, 1962); B. *Nautilus* (modified after Naef, 1921-1923); C. *Sepia* (modified after Naef, 1921-1923); D. *Loligo* (modified after Naef, 1921-1923); E. Incirrate octopod (modified after Naef, 1921-1923). (Aff. Br. V., afferent branchial vessel; Br. Can., branchial canal; Br. Gl., branchial gland; Cart. supp., cartilagenous supporting rod; Br. Gl., branchial gland; Eff. Br. V., efferent branchial vessel; Musc. Bas., muscular base).

Character No. 30: Right oviduct. Character states: 0 - absent; 1 - present.
Comments. The oviducts are gonoducts that open into the visceropericardial coelom and exit into the mantle cavity (Figs. 1A, B, D). In coleoids the left, but not the right, oviduct is always present. "Present" refers to physical presence irrespective of functionality.

Character No. 31: Oviducal gland symmetry. Character states: 0 - radial symmetry; 1 - bilateral symmetry; 2 - asymmetry.
Comments. The oviducal glands are organs that surround the oviducts and contain numerous glandular lamellae that are involved in secretion of egg cases or masses. The oviducal gland of *Vampyroteuthis* surrounds the oviducal opening and consists of two thick, contiguous, equal-sized rings. Each ring is composed of flattened lamellae. Typically the leaflets of the outer ring which are attached only proximally are freely exposed to the mantle cavity. A circular membrane that covers the more proximal ring, however, is capable of expanding to cover the outer ring. The entire organ is radially symmetrical (Fig. 12B). The double nature of the gland is characteristic of coleoids. In decapods the proximal portion is much smaller and both proximal and distal portions are bilaterally symmetrical (Fig. 12C) while in octopods the distal portion is often smaller and the glands

are radially symmetrical. The oviducal gland of *Nautilus* forms the terminal portion of the oviduct. It appears to be highly glandular and has thick folds or lamellae. The gland, in our poorly preserved specimens, has a slit-like opening and the arrangement of lamellae shows it to be asymmetrical (Fig. 12A).

Character No. 32: Oviducal gland position. Character states: 0 - gland terminal (located at end of oviduct); 1 - gland subterminal (oviduct continues distal to gland).
Comments. Oviducal glands are located either at the opening of the oviduct (Figs. 1A, D) or well proximal to the oviduct external opening which thereby defines a distinct distal oviduct (Fig. 1B). A distal oviduct can be identified by the lack of glandular leaflets and often lacks a circular orifice. In *Sepiola* the distal portion of the oviducal gland is very slender and elongate and gives the false impression of a distal oviduct.

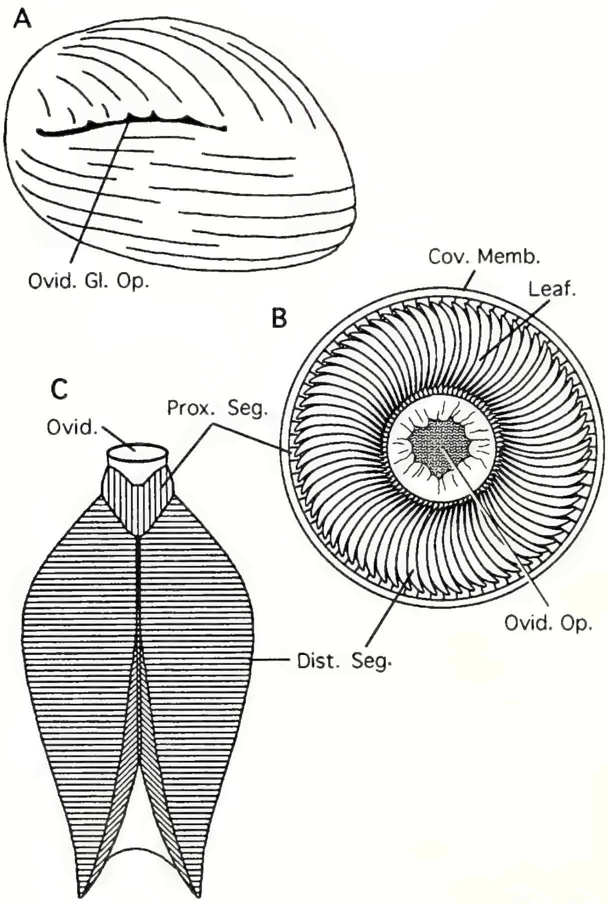

Fig. 12. Oviducal glands (diagramatic). A. *Nautilus*. B. *Vampyroteuthis*. C. *Abralia*. (Cov. Memb., covering membrane; Dist. Seg., distal segment of oviducal gland; Leaf., leaflet; Ovid., oviduct; Ovid. Gl. Op., oviducal gland opening; Ovid. Op., oviduct opening; Prox. Seg., proximal segment of oviducal gland).

Character No. 33: Photosensitive vesicles. Character states: 0 - within cephalic cartilage; 1 - above funnel; 2 - on stellate ganglia.
Comments. The photosensitive vesicles are vesicular organs that function in detection of light for a variety of purposes. In decapods they occupy a variety of locations but all are within the region of the head bounded by the cephalic cartilage (Fig. 3B). In octopods they lie on the stellate ganglia and in *Vampyroteuthis* they lie just above the dorsal surface of the funnel (Fig. 3B). Photosensitive vesicles have never been described in *Nautilus*. This is an unambiguous character that has been previously described in most families of concern here.

Character No. 34: Inferior frontal lobe system of the brain. Character states: 0 - absent; 1 - partially present; 2 - present.
Comments. The inferior frontal lobe system of octopods consists of the inferior frontal, subfrontal, and posterior buccal lobes (J. Z. Young, 1971). In decapods this entire system is represented by the posterior buccal lobes (often called the inferior frontal lobes) (J. Z. Young, 1988). In *Vampyroteuthis* the complex connections posteriorly from the posterior buccal lobe (*sensu lato*) and the central region of the supraesophageal mass is much more complex than in decapods and J. Z. Young (1977: 385) suggested it "may represent a poorly differentiated subfrontal lobe." He considered this and some differentiation of the dorsal part of the buccal lobe which he interpreted as an inferior frontal lobe, to be a stage of "incipient development of an apparatus for more elaborate processing of tactile information... ." We agree with this interpretation and coded this character as a separate state for *Vampyroteuthis* that is intermediate between the octopod and decapod conditions. This transformation series is best defined as "ordered." The construction of the brain of *Nautilus* is very different from coleoids and cannot be coded for this character.

Character No. 35: Head-mantle fusion: arm-base-to-anterior-mantle muscle. Character states: 0 - present; 1 - absent.
Comments. In a variety of coleoids the dorsal surface of the mantle has become fused to the head. The variation in the details of the fusion indicate that fusion has occurred several times during evolution. Character 35 describes a characteristic of the octopod fusion in which muscles attach at the junction of the dorsal arm bases and on the anterior edge of the dorsal mantle (Fig. 10B). As a result, it lumps all other families together under state 1 (absent). This is acceptable at our present level of analysis because state 1 is the plesiomorphic state in the Coleoidea. Even though *Nautilus* lacks a mantle-propulsion system, we consider its

lack of head-mantle fusion a relevant state.

Character No. 36: Arm III hectocotylization. Character states: 0 - absent; 1 - present.
Comments. Hectocotylization refers to the modification of an arm in males for the transfer of sperm to the female (Fig. 10B). Arm III in octopods and vampyromorphs could be the homologue of arm IV (tentacle) in decapods (see Character 6). For this character we have not made this assumption and compared the morphological arm III in all groups. The distribution of this character, however, would be the same for either interpretation of arm relationships. Either one or both members of an arm pair could be modified. Because *Nautilus* lacks specific homologues to each of the ten arms of coleoids, it has no relevant character state.

Character No. 37: Arm V hectocotylization. Character states: 0 - absent; 1 - present.
Comments. We assume that the hectocotylization of different arm pairs (cf. Character 36) represents independent evolutionary events and, therefore, qualify as separate characters.

Character No. 38: Collagenous tunics on mantle. Character states: 0 - absent; 1 - present.
Comments. Many decapods contain a collagenous tunic over the mantle that, apparently, acts to resist change in mantle length during the jet cycle (Ward and Wainwright, 1972). To evaluate this character, we have looked for a continuous connective tissue sheath over the inner and outer surfaces of the mantle muscle in histological sections. However, this approach did not always provide unambiguous results. Connective tissue is present on the mantle of all cephalopods and the difference between a continuous, uniform "tunic" and an irregular layer was not always clear. Indeed, we were unable to clearly distinguish what could be intermediate states in *Sepia* and *Octopus*. Because *Nautilus* lacks a mantle-propulsion system, a necessary precursor to the development of a collagenous tunic, it has no relevant character state.

Character No. 39: Spermatophores with ejaculatory apparatus. Character states: 0 - present; 1 - sperm packets; 2 - encapsulated coil.
Comments. All coleoid cephalopods except the finned octopods have characteristic spermatophores that contain a complex ejaculatory apparatus (Figs. 13A, B, D). The finned octopods produce peculiar sperm packets (Fig. 13E). This one character probably represents a suite of modifications to the spermatophore and the glands that form them, setting the cirrates apart from other coleoids. *Nautilus* has a spermatophore that lacks an ejaculatory apparatus (Mikami and Okutani, 1981), is very different from that of cirrate octopods (Fig. 13C), and has been given a separate character state (state 2).

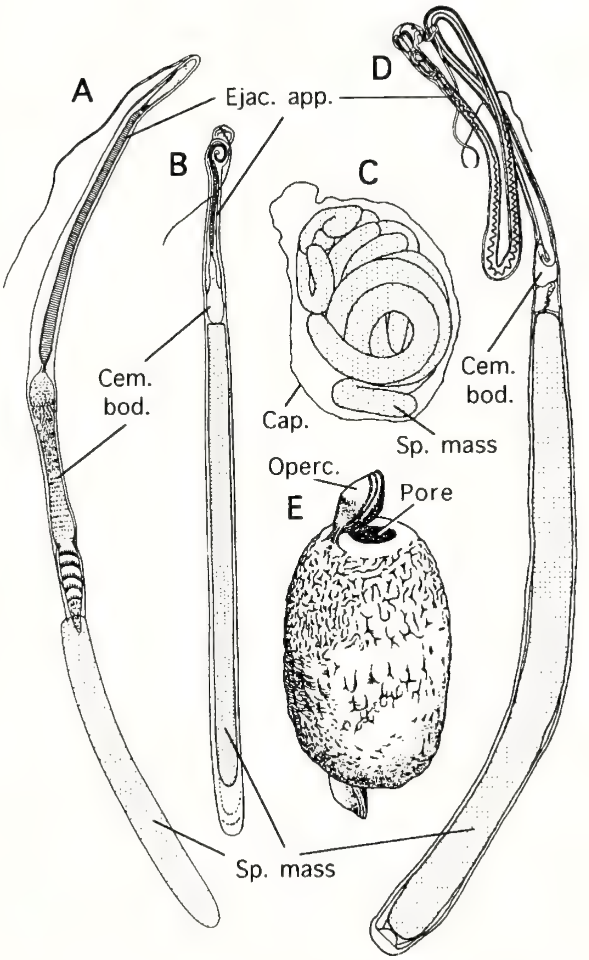

Fig. 13. Spermatophores. A. *Eledone* (modified after Marchand, 1912). B. *Loligo* (modified after Marchand, 1912). C. *Nautilus* (modified after Mikami and Okutani, 1981). D. *Vampyroteuthis* (modified after Hess, 1987). E. *Opisthoteuthis* (after Villanueva, 1992). (Cap., capsule; Cem. bod., cement body; Ejac. app., ejaculatory apparatus; Operc., operculum; Sp. mass, sperm mass).

Character No. 40: Superior buccal lobe. Character states: 0 - widely separated from brain; 1 - adjacent to brain; 2 - fused to brain.
Comments. The position of the superior buccal lobe relative to the supraesophageal mass of the brain varies greatly among different cephalopods depending largely on the distance between the buccal mass and the brain (Fig. 5). There are, however, three distinct states as defined above. State 0 is found in decapods and *Nautilus* (Figs. 5A, B), state 1 in *Vampyroteuthis* (Fig. 5C), and state 2 in octopods (Fig. 5D). J. Z. Young (1988: 255) misstated the condition in *Vampyroteuthis* as "the superior buccal lobes are joined

with the rest of the brain as in octopods" due to his examination of a distorted specimen (note the 90° turn in the esophagus in his fig. 5) that artificially compressed the superior buccal mass against the brain. In normal specimens the lobes are clearly separated medially. As a result we have given *Vampyroteuthis* a separate state which is intermediate between the decapod and octopod states and consider this character to have an "ordered" transformation series. A similar situation, with the exception of *Nautilus*, occurs with respect to the brachial lobe, but as this character is probably not independent from Character 40 in the coleoids, it was not used.

Character No. 41: Horizontal arm septa. Character states: 0 - absent; 1 - present.
Comments. Cirrate octopods have a horizontal septum that inserts on the circular muscle layer that forms the outer and thinner portion of the cyclindrical muscular wall of the arm. It is orally concave in cross-section and divides the muscular tube within each arm into oral and aboral regions. *Japetella* (Bolitaenidae) has a similar septum but its insertion on two membranes, extending in an oral-aboral plane well internal to the arm muscles, suggests that the cirrate and bolitaenid structures are derived independently.

Character No. 42: Paired digestive gland duct appendages. Character states: 0 - single; 1 - paired.
Comments. The digestive gland duct appendages (DGDA) are glands that attach to the ducts of the digestive glands. In decapods they are spread along the long ducts to form paired, multilobed structures (Figs. 8E, F) while in *Vampyroteuthis* and the octopods they are compacted next to the digestive gland in a single (unpaired) structure (Figs. 8B, D, 9). Although there is some variation in the Oegopsida, the organs always remain paired even where occasionally compacted against the digestive gland. Because *Nautilus* lacks DGDA, it has no relevant character state.

Character No. 43: Relative positions of the DGDA and the nephridial coelom. Character states: 0 - DGDA lies in the nephridial coelom; 1 - DGDA not in the nephridial coelom.
Comments. The DGDA can be suspended within a branch of the nephridial coelom and intimately covered by the coelomic lining (Figs. 8E, F) or lie outside the coelom and separated by, at least, several tissue layers from the coelom (Figs. 8B, D, 9). *Nautilus* lacks a relevant character state.

Character No. 44: Head width index. Character states: 0 - 0.49; 1 - 0.5-0.99; 2 - 1.0-1.49; 3 - 1.5-1.99; 4 - 2.0-2.49; 5 - 2.5-2.99; 6 - 3.0-3.49; 7 - 3.5-3.99; 8 - 4.0-4.49; 9 - 4.5-4.99.
Comments. This character relates head width to eye size by measuring the number of eye radii that separate the eyes. States were assessed by measuring the head width and the lens diameter. The eye radius was then calculated and doubled for subtraction from the head width. This is a continuous character that has been converted to a discrete character by dividing the range of the character into equal segments (Fig. 14). The definitions were compromised in groups (some sepioids and cirrate octopods) with a dorsal tilt to the eyes, and therefore, this character was excluded from the analysis.

Character No. 45: Gill filament attachment. Character states: 0 - inner and outer filaments free; 1 - outer filaments attached; 2 - inner and outer filaments attached.
Comments. In coleoids the gill filaments often are attached to the gill base surrounding the branchial gland by triangular membranes that are supported by slender cartilaginous rods (Figs. 11A, C, D). The length of the rod determines the distance of the filament from the branchial gland. The length of the rod varies with the position along the gill, the side of the gill, and the taxon. When the rod is absent in coleoids, the filament is defined as "attached" (Fig. 11E) and, in some cases, the difference between attached and free is slight. The gills of *Nautilus* are comparable to those of coleoids but lack a branchial gland and supporting rods to the filaments (Fig. 11B). The muscular triangular membranes broadly separate the tips of the filaments from the muscular base that occupies the position of the branchial

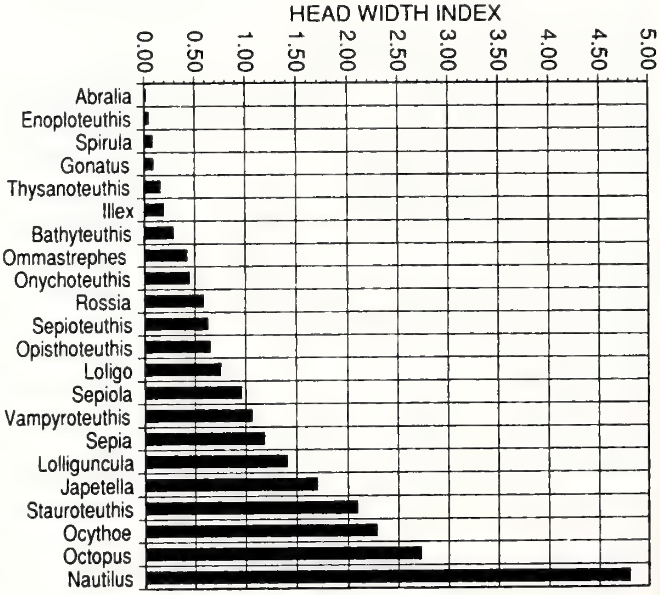

Fig 14. Histogram showing head-width index values for 22 taxa (Character 44).

gland in coleoids. As a result, we coded *Nautilus* as state 0. We had difficulty evaluating the character states in preserved animals and have concerns whether the states are adequately defined. While our attempt to determine and survey states is presented in the data matrix, we excluded this character from the analysis.

Character No. 46: Digestive gland. Character states: 0 - multiple digestive glands; 1 - two separate digestive glands (unipaired); 2 - digestive glands fused (single gland).
Comments. In coleoids the digestive gland consists of either two separate lobes or a single lobe. Separate but adjacent digestive glands can appear to be a single organ but are separated by a membrane and, in fresh specimens, can be pulled apart without damage to either organ. The definition of "separate" requires the lobes to be separate over their entire length. Within the coleoids, the appearance of two anlagen of the digestive gland in the embryos of *Loligo* and the presence of two digestive gland ducts in all coleoids indicate that unipaired glands (state 2) is the plesiomorphic state.

Character No. 47: Longitudinal muscle on mantle. Character states: 0 - present; 1 - absent.
Comments. We systematically sectioned the mantle in the region of the mantle-locking apparatus for the presence of a layer of longitudinal muscles. Generally a thin layer of longitudinal muscles was present external to the circular muscles on the outer surface. The longitudinal layer, however, is often discontinuous at least in the region surveyed and in a few families was absent.

Character No. 48: Position of vena cava relative to intestine. Character states: 0 - intestine passes ventral to vena cava; 1 - intestine passes dorsal or anterior to vena cava.
Comments. The cephalic vein extends posteriorly from the head along or near the ventral surface of the visceral mass until it splits to form the two branches of the vena cava. The intestine, arising from the visceral nucleus passes dorsal or anterior to this bifurcation (Figs. 8E, F) or, in some cases, passes posterior then ventral to the bifurcation and, as a result, traps the vena cava within the U-shape of the digestive tract (Figs. 8A, B, 9). In preserved specimens the cephalic vein and vena cavae often have collapsed and cannot be followed. As a result, we generally used the position of the intestine relative to the position of the renal appendages as an indicator of the character. In *Nautilus* the terminal portion of the intestine lies on the ventral mantle wall rather than on the visceral mass and is thereby separated from the vena cava (Griffin, 1900). As a result, this character is not applicable to *Nautilus*.

Character No. 49: Posterior salivary gland. Character states: 0 - absent; 1 - posterior to brain; 2 - on or in buccal mass.
Comments. The posterior salivary glands generally lie posterior to the cephalic cartilage. However, Aldred *et al.* (1983) found that in *Cirrothauma* a single gland is present and it lies within the buccal mass. Ebersbach (1915) reported a similar situation for *Grimpoteuthis umbellata* (Fischer, 1883) (his *Cirroteuthis umbellata*, see systematic comments in Voss, 1988). This character was surveyed as a possible synapomorphy for the cirrates. *Nautilus* lacks posterior salivary glands.

Character No. 50: Position of gonad relative to the VP coelom. Character states: 0 - gonad mostly within the coelom; 1 - less than 50% within.
Comments. In most cephalopods, the gonad, except for its attachment sites, lies mostly within the VP coelom, that is, it is covered by the coelomic lining (Figs. 1A, D). In octopods, however, the gonad lies mostly outside the coelom in a gelatinous milieu (Figs. 1B, C, E). Unfortunately, we had difficulty in incirrate octopods in determining how much of the gonad was covered by coelomic lining although it appeared to be greater than 50%.

In animals as complex as cephalopods numerous additional characters that have potential phylogenetic value at this level remain to be identified and surveyed. We mention four that we were unable to survey.

(1) Funnel-locking apparatus (Fig. 10A). In many cephalopods a specialized locking apparatus locks the funnel to the inner surface of the mantle. The locking apparatus occurs in decapods and some octopods and is probably convergent in these two groups. We suspect that in decapods the funnel component has a cartilaginous base that is lacking in octopods. However, even if this proves to be a valid distinction, it cannot be polarized by the condition in *Nautilus*. We originally attempted to apply this definition to the mantle component of the locking apparatus but found a large variety of structures with cartilage only rarely being present.

(2) Suprabrachial commissure (Fig. 5D). Most octopods have a strong commissure that loops dorsal to the esophagus and connects the lateral regions of the left and right brachial lobes of the subesophageal region of the brain (Aldred *et al.*, 1983). In cirrate octopods this commissure lies beneath the posterior buccal lobe but is separated from it by connective tissue. A corresponding commissure exists in *Vampyroteuthis* but is small and lies just beneath the posterior buccal lobe (pers. obs.). A counterpart is unknown in the decapods. Surprisingly this commissure appears to be lacking in *Japetella* (J. Z. Young, 1977). Unfortunately this is another character that cannot be polar-

Table 2. Data matrix for 50 characters and 17 taxa. Asterisk indicates character used in this study; slash indicates two character states present.

	1-5					6-10					11-15					16-20					21-25				
				*		*	*	*	*	*							*	*		*	*				*
Bathyteuthidae	0	0	1	0	2	0	1	2	0	1	2	1	1	1	1	0	0	0	1	0	0	1	1	1	1
Enoploteuthidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	0	1	1	1
Gonatidae	0	0	1	0	2	0	1	2	0	1	2	2	0	1	0	0	0	0	1	0	0	1	1	1	1
Loliginidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	1	1
Ommastrephidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	1	1
Onychoteuthidae	0	0	1	0	2	0	1	2	0	1	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1
Sepiidae	1	0	0	0	2	0	1	2	0	1	2	2	0	1	1	0	0	0	1	0	0	1	1	0	1
Sepiolidae	0	0/1	1/3	0/4	2	0	1	2	0	1	1/2	1/2	1	0	0/1	0	0	0	1	0	0	1	1	0	1
Spirulidae	1	1	0	2	2	0	1	2	0	1	2	2	1	1	1	0	0	0	1	0	0	1	1	0	3
Thysanoteuthidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	1	1
Bolitaenidae	0	1	3	4	0	2	0	0	2	0	0	0	1	0	1	1	2	1	0	1	1	0	0	1	2
Octopodidae	0	1	2	3	0	2	0	0	2	0	0/1	0/1	1	0	1	1	2	1	0/1	1	1	0	0	1	2
Ocythoidae	0	1	3	4	0	2	0	0	2	0	1	1	1	0	0	0	2	1	0	1	1	0	0	1	2
Stauroteuthidae	0	1	2	1	0	2	0	0	2	0	0	0	0	0	1	1	1	1	0	1	1	0	0/1	2	2
Opisthoteuthidae	0	1	2	1	0	2	0	0	2	0	0	0	0	0	1	1	1	1	0	1	1	0	0	2	1/2
Vampyroteuthidae	1	0	1	0	0	1	0	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	0
Nautilidae/Ancestor	1	?	0	?	1	0	0	?	?	?	?	?	?	?	?	?	?	0	0	0	0	1	0	0	?

	26-30					31-35					36-40					41-45					46-50				
	*		*	*		*	*	*	*	*	*			*	*	*		*					*		
Bathyteuthidae	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	2	0	1	1	0
Enoploteuthidae	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0	2	0	1	1	0
Gonatidae	0	0	0	0	1	1	0	0	0	1	0	1	1	?	0	0	?	0	0	0	2	1	1	1	0
Loliginidae	0	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	1	0	1/2	0	2	0	0	1	0
Ommastrephidae	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0/1	2	1	1	1	0
Onychoteuthidae	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	2	0	1	1	0
Sepiidae	0	0	0	1	0	1	0	0	0	1	0	1	?	0	0	0	1	0	2	0	1	0	0	1	0
Sepiolidae	1	0	0/1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	2	0	0	1	0
Spirulidae	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0
Thysanoteuthidae	0	0	0	0	1	1	0	?	?	1	0	1	1	0	?	0	1	0	0	0	2	1	1	1	0
Bolitaenidae	1	1	1	2	1	0	1	2	2	0	1	0	0	?	2	0	0	1	3	2	2	0	1	1	0
Octopodidae	1	1	1	2	1	0	1	2	2	0	1	0	?	0	2	0	0	1	5	2	2	0	1	1	0
Ocythoidae	1	1	1	2	1	0	1	2	2	0	1	0	0	0	2	0	0	1	4	1	2	0	1	1	0
Stauroteuthidae	1	1	1	0	0	0	1	2	2	0	0	0	0	1	2	1	0	1	4	0	2	0	1	2	1
Opisthoteuthidae	1	1	1	0	0	0	1	?	2	0	0	0	0	1	2	1	0	1	?	2	2	?	1	0	1
Vampyroteuthidae	2	0	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	2	0	2	0	0	1	0
Nautilidae/Ancestor	?	0	?	?	1	2	0	?	?	1	?	0	?	2	0	0	?	?	9	0	0	0	?	0	0

ized by *Nautilus*.

(3) Genital pocket. The genital pocket is an invagination of the integument lining the mantle cavity that surrounds the accessory spermatophore organs. Absence of a genital pocket in male cirrates could be a synapomorphy for this group. This is one of a probable suite of characters associated with the degeneration of spermatophore structure.

(4) Dorsal lobe of the nephridial sacs (Figs. 8A-D). These lobes (see Character 19) could prove to be a synapomorphy of the Vampyromorpha and Octopoda but are difficult to survey in preserved material.

ANALYSIS

We surveyed 50 characters in 17 families and these data are presented in Table 2. We eliminated four of these characters (Nos. 13, 38, 44, 45) from the analysis because of potential errors related to questionable definition of character states or accuracy of surveying the states. We also eliminated seven characters (Nos. 1, 5, 22, 23, 27, 30, 47) after determining that the plesiomorphic state was "present" and the apomorphic state was "absent." For these characters we could not determine if losses represented homology or involved homoplasy. Polarity was determined for these by outgroup comparison on MacClade. Some other characters fell so obviously into this pattern (*e. g.* presence/absence of fins) that they were not surveyed. Characters with "fused" as the apomorphic state also presented a problem in distinguishing between homology and homoplasy. Three characters (Nos. 19, 42, 46) were eliminated on this basis as their plesiomorphic condition was the unfused state (polarity for Character 19 based on outgroup condition and for 46 on ontogeny). We further eliminated

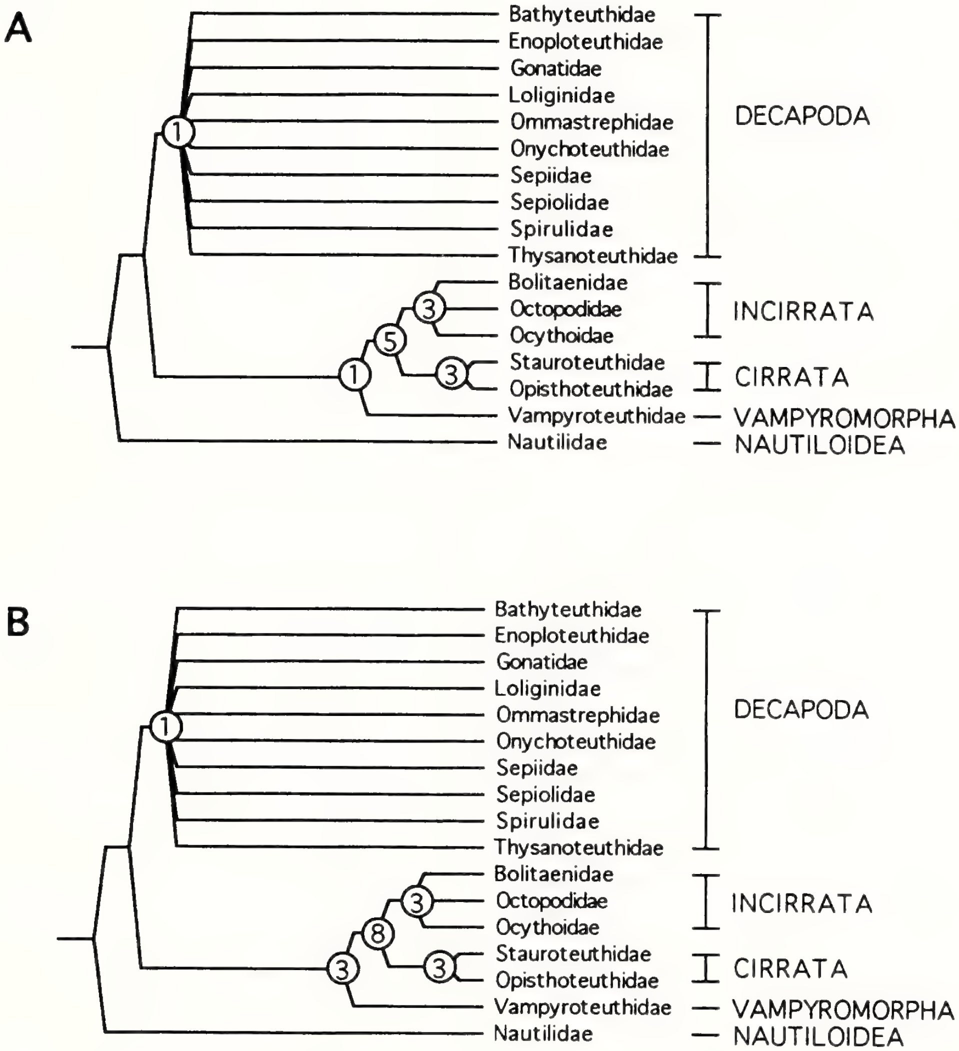
A
Bathyteuthidae
Enoploteuthidae
Gonatidae
Loliginidae
Ommastrephidae
Onychoteuthidae
Sepiidae
Sepiolidae
Spirulidae
Thysanoteuthidae
Bolitaenidae
Octopodidae
Ocythoidae
Stauroteuthidae
Opisthoteuthidae
Vampyroteuthidae
Nautilidae
DECAPODA
INCIRRATA
CIRRATA
VAMPYROMORPHA
NAUTILOIDEA
1
3
5
1
3
B
Bathyteuthidae
Enoploteuthidae
Gonatidae
Loliginidae
Ommastrephidae
Onychoteuthidae
Sepiidae
Sepiolidae
Spirulidae
Thysanoteuthidae
Bolitaenidae
Octopodidae
Ocythoidae
Stauroteuthidae
Opisthoteuthidae
Vampyroteuthidae
Nautilidae
DECAPODA
INCIRRATA
CIRRATA
VAMPYROMORPHA
NAUTILOIDEA
1
3
8
3
3

Fig. 15. Strict consensus of shortest trees from PAUP search of data set with 25 characters (14 trees; tree length 46). Numbers at internal nodes represent Support Indices. Indices of shortest trees: consistency index 0.98; homoplasy index 0.09; retention index 0.99; rescaled consistency index 0.97. Mickevich's consensus information from the consensus tree = 0.312. A. All character states unordered (Analysis I). B. Three characters with states ordered, others unordered (Analysis IIA). Bootstrap values (1,000 replicates) for nodes are: decapod node 70, octopod/vampire node 98, octopod node 100, incirrate node 97, cirrate node 97.

three characters (Nos. 2, 3, 50) because they might not be completely independent from other characters (*i. e.* Nos. 4, 4, 20, respectively) due to some overlap in definitions. One character (No. 37) was eliminated as it varied only within a major group (Decapoda) and was, therefore, uninformative at the level of interest in this study. Seven characters (Nos. 11, 12, 14, 15, 16, 24, 48) exhibited homoplasy under all plausible phylogenetic rearrangement of the groups. The worst of these was Character 24 (branchial canal), a presence/absence character with "absence" in *Nautilus* presumably defining the plesiomorphic state. All rearrangements resulted in multiple homoplasy, varying from three independent derivations to three independent losses of branchial canals with the minimum homoplasy involving two convergences. Until these seven characters can be redefined to eliminate homoplasy, we eliminate them from analyses. This left 25 characters.

We first analyzed the data (Analysis I, PAUP, Fig. 15A) with all characters entered as "unordered." To determine the strength of the nodes we determined the support index (SI) for all internal nodes (Eernisse and Kluge, 1993). Decapod monophyly (SI = 1) was supported unambiguously by only a single character (No. 7, Arms IV). However, eight other characters that we were not able to polarize (Nos. 8, Sucker rings; 9, Sucker stalks; 10, Sucker symmetry; 17, Fin cartilage; 31, Oviducal gland symmetry;

33, Photosensitive vesicles; 34, Subfrontal lobes; 43, DGDA/nephridial coeloms) have states that are, presently, found exclusively in the decapods and in all the decapods. In this first analysis only a single unambiguous character (No. 18, Statocyst outer capsule) change supported the vampyromorph/octopod node (SI = 1). Four additional characters (Nos. 10, Sucker symmetry; 17, Fin cartilage; 31, Oviducal gland symmetry; 43, DGDA/nephridial coeloms) could support this clade depending on which states prove to be plesiomorphic within coleoids. Monophyly of the Octopoda (SI = 5) was supported by five characters (Nos. 20, Visceropericardial coelom; 21, Dorsal mantle cavity; 28, Nuchal cartilage; 32, Oviducal gland position; 35, Arm-mantle muscle) and, potentially seven more (Nos. 6, Arms II; 8, Sucker rings; 9, Sucker stalks; 25, Mantle septum; 33, Photosensitive vesicles; 34, Subfrontal lobe; 40, Superior buccal lobe) depending on how polarity is resolved. The Cirrata (SI = 3) were supported by unambiguous changes in three characters (Nos. 39, Spermatophores; 41, Horizontal arm septa; 49, Posterior salivary glands) and one potential unpolarized character (No. 4, Shell shape). The Incirrata (SI = 3) were supported by unambiguous changes in three characters (Nos. 17, Fin cartilage; 29, Cornea; 36, Arm III hectocotylus) although the first is a "non-applicable" state resulting from fin loss.

We next analyzed the data (Analysis IIA, PAUP, Fig. 15B) after ordering three characters (Nos. 6, Arms II; 34, Subfrontal lobe; 40, Superior buccal lobe) whose evidence warrants this restriction (see Comments under each character). We consider this to be our best estimate of the phylogeny at this time. Topology and tree length did not change from the previous analysis. However support for some clades improved. Support for the decapod clade was unchanged, while the SI for the vampyromorph/octopod node increased to 3 from the additional unambiguous changes in two characters (Nos. 6, 40). Character 34 did not contribute to this node due to its ambiguous polarization at the coleoid node. All three ordered characters supported additional unambiguous character changes for the Octopoda which increased the SI to 8. Support for the Cirrata and Incirrata were unchanged. In both analyses the low degree of homoplasy in the data set utilized apparently resulted in the SI being identical to the number of unambiguous character changes at each node. We ran this same data set in Hennig86 (Analysis IIB) which provided the same topology for the six internal nodes (14 trees, tree length = 46, consistency index = 0.97, retention index = 0.99).

DISCUSSION

Analysis II provided marginal support for monophyly of the decapod clade (one character). However, the large pool of characters with states diagnostic of decapods but unpolarized suggests that additional support is likely. The vampyromorph/octopod clade seems reasonably well supported by three characters although some of these carry assumptions. Four unpolarized characters offer the possibility of additional support. The monophyly of the Octopoda was very well supported by eight characters with possible additional support from another three unpolarized characters.

Although monophyly of decapods, cirrate and incirrate octopods (Vampyromorpha is monotypic) was supported, we emphasize that not all families were included in our examination of these higher taxa. While we expect these higher taxa were adequately representated, confirmation from all families is needed.

The tree resulting from Analysis II rests on only 16 characters involving 18 character state changes. Ten characters defined the decapod, octopod, and octopod + vampyromorph clades. An additional six characters defined the two octopod clades. The remaining nine characters were effectively neutral (*i. e.* they neither added to nor substracted from support for the internal nodes). One of our primary obstacles in this study has been our inability to polarize many of the characters at the basal coleoid node. Because of this problem, eight of the nine neutral characters were "neutralized" and six of the other characters were only partially effective. As expected, PAUP analysis of the 16 characters gave the identical tree with identical SI values to that of Analysis II (tree length, of course, was shorter: 24) while analysis of the nine neutral characters resulted in a completely unresolved consensus tree.

In future analyses of decapod families with more nodes and a more complex hierarchy of nodes, emphasis must be placed on thoroughly understanding the interrelationships of characters that will enable determination of the basal clades upon which much of the polarization at more resolved nodes must rest. Efforts should, therefore, be placed on locating characters that have counterparts in outgroups (or can be polarized by ontogeny/paleontology), and that do not involve loss as the synapomorphy. Multistate characters in which evolutionary pathways can be reconstructed would be especially valuable. Homoplasy in these "basal" characters is dangerous but elimination of homoplasic characters is not only wasteful of any useful information they can contain but could be impossible, in a more complex and poorly understood phylogeny, by the method used here (*i. e.* recognition of which characters are homoplastic on the basis of "implausible relationships"). Only a thorough understanding of the relationships among character states through detailed study can guard against this pitfall. We simply argue in favor of an approach to phylogenetic studies by the methodology referred to by Mickevich (1995) as "synapomorphic cladistics" and long-recognized by many others (*e. g.* Bryant, 1989).

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LITERATURE CITED

Aldred, R. G., M. Nixon, and J. Z. Young. 1983. *Cirrothauma murrayi* Chun, a finned octopod. *Philosophical Transactions of the Royal Society of London,* series B 301:1-54.

Berthold, T. and T. Engeser. 1987. Phylogenetic analysis and systematization of the Cephalopoda (Mollusca). *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* (NF) 29:187-220.

Bizikov, V. A. 1991. *Squid Gladius: Utilization for Study of Growth, Age, Internal Structural Aspects and Evolution (Ommastrephidae).* Doctoral Dissertation, Academy of Science USSR, P.P. Shirshov Institute of Oceanology. 27 pp.

Boletzky, S. v. 1978-1979. Nos connaissances actuelles sur le développement des Octopodes. *Vie et Milieu* 28-29(1AB):85-120.

Boletzky, S. v. 1993a. Development and reproduction in the evolutionary biology of Cephalopoda. *GeoBios* 15:33-38.

Boletzky, S. v. 1993b. The arm crown in cephalopod development and evolution: a discussion of morphological and behavioral homologies. *American Malacological Bulletin* 10:61-69.

Bonnaud, L., R. Boucher-Rodoni, and M. Monnerot. 1994. Phylogeny of decapod cephalopods based on partial 16S rDNA nucleotide sequences. *Comptes Rendus des Sciences, Série III, Sciences de la Vie* 317:581-588.

Brakoniecki, T. F. 1986. *A Generic Revision of the Family Loliginidae (Cephalopoda: Myopsida) Based Primarily on the Comparative Morphology of the Hectocotylus.* Doctoral Dissertation, University of Miami, Coral Gables, Florida. 164 pp.

Bryant, H. N. 1989. An evaluation of cladistic and character analyses as hypothetico-deductive procedures, and the consequences for character weighting. *Systematic Zoology* 38:214-227.

Clarke, M. R. 1988. Evolution of recent cephalopods - a brief review. *In: The Mollusca, 12: Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 331-340. Academic Press, New York.

Clarke, M. R. and L. Maddock. 1988. Statoliths from living species of cephalopods and evolution. *In: The Mollusca, 12: Paleontology and Neontology of Cephalopods*, M. R. Clarke and E. R. Trueman, eds. pp. 169-184. Academic Press, New York.

Donovan, D. T. 1977. Evolution of the dibranchiate Cephalopoda. *Symposia of the Zoological Society of London* 38:15-48.

Ebersbach, A. 1915. Zur Anatomie von *Cirroteuhtis umbellata* Fischer und *Stauroteuthis* sp. *Zeitschrift für Wissenschaftliche Zoologie* 113:361-483.

Eernisse, D. J. and A. G. Kluge. 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. *Molecular Biology and Evolution* 10:1170-1195.

Farris, J. S. 1988. *HENNIG86 Reference. Version 1.5.* James S. Farris.

Fioroni, P. 1981. Die Sonderstellung der Sepioliden, ein Vergleich der Ordnungen der rezenten Cephalopoden. *Zoologische Jahrbücher Abteileng für Systematik Oekologie und Geographie der Tiere* 108:178-228.

Graziadei, P. P. C. 1971. The nervous system of the arms. *In: The Anatomy of the Nervous System of* Octopus vulgaris, J. Z. Young, ed. pp. 44-61. Claredon Press, Oxford.

Griffin, L. E. 1900. The anatomy of *Nautilus pompilius. Memoirs of the National Academy of Sciences, Washington* 8:101-197.

Hillig, R. 1913. Das Nervensystem von *Sepia officinalis. Zeitschrift für Wissenschaftliche Zoologie* 101:736-800.

Hess, S. C. 1987. *Comparative Morphology, Variability and Systematic Applications of Cephalopod Spermatophores (Teuthoidea and Vampyromorpha).* Doctoral Dissertation, University of Miami, Coral Gables, Florida. 580 pp.

Hunt, S. and M. Nixon. 1981. A comparative study of protein composition in the chitin-protein composition complexes of the beak, pen, sucker disc, radula and oesophageal cuticle of cephalopods. *Comparative Biochemistry and Physiology* 68B:535-546.

Isgrove, A. 1909. *Eledone. Liverpool Marine Biology Committee, Memoirs on Typical British Marine Life & Animals 18.* The University Press of Liverpool. 105 pp.

Khromov, D. N. 1990. Cuttlefishes in the systematics and phylogeny of Cephalopoda. *All-Union Research Institute of Marine Fishery and Oceanography* 69(2):12-20.

Maddison, W. P. and D. R. Maddison. 1992. *MacClade: Analysis of Phylogeny and Character Evolution. Version 3.0.* Sinauer Associates, Sunderland, Massachusetts.

Mangold, K. and A. Portmann. 1989. *Traité de Zoologie, Tome V. Céphalopodes.* Masson, Paris. 804 pp.

Marchand, W. 1912. Studien über Cephalopoden II. Ueber die Spermatophoren. *Zoologica* 67:171-200.

Mickevich, M. F. 1995. Cladistic analysis today (abstract). *Program and Abstracts, 61st. Annual Meeting, American Malacological Union, University of Hawai'i at Hilo,* p. 34.

Mikami, S. and T. Okutani. 1981. A consideration on transfer of spermatophores in *Nautilus macromphalus. Venus* 40(1):57-62.

Naef, A. 1921-1923. Cephalopoda. *Fauna e Flora del Golfo di Napoli Monograph, 35.* Translated from German, Israel Program for Scientific Translations. 863 pp.

Naef, A. 1928. Die Cephalopoden. 1 Teil, 2 Band. Embryologie. *Fauna e Flora del Golfo di Napoli. Monograph 35.* Stazione Zoologica di Napoli, Naples. 322 pp.

Nesis, K. N. 1987. *Cephalopods of the World: Squids, Cuttlefishes, Octopuses, and Allies.* T. F. H. Publications, Neptune City, New Jersey. 351 pp.

Nixon, K. C. and J. M. Carpenter. 1993. On outgroups. *Cladistics* 9:413-426.

Nixon, M. and P. N. Dilly. 1977. Sucker surfaces and prey capture. *Symposia of the Zoological Society of London* 38:447-512.

Pickford, G. E. 1940. The Vampyromorpha, living-fossil Cephalopoda. *Transactions of the New York Academy of Sciences* (ser. 2) 2(7):169-181.

Pickford, G. E. 1946. *Vampyroteuthis infernalis* Chun. An archaic dibranchiate cephalopod. I. Natural history and distribution. *Dana Report* 29:1-40.

Pickford, G. E. 1950. The Vampyromorpha (Cephalopoda) of the Bermuda Oceanographic Expedition. *Zoologica* 35(1):87-95.

Platnick, N. I., C. E. Griswold, and J. A. Coddington. 1991. On missing entries in cladistic analysis. *Cladistics* 7:337-344.

Robson, G. C. 1932. *A Monograph of the Recent Cephalopoda Based on the Collections of the British Museum (Natural History). Part II. The Octopoda (Excluding the Octopodinae).* British Museum, London. 359 pp.

Roper, C. F. E., M. J. Sweeney, and C. E. Nauen. 1984. FAO species catalogue: cephalopods of the world. *FAO Fisheries Synopsis* 3(125):1-277.

Rudall, K. M. 1955. The distribution of collagen and chitin. *Symposia of the Society of Experimental Biology* 9: 49-71.

Swofford, D. L. 1993. *PAUP: Phylogenetic Analysis using Parsimony. Version 3.1.1.* Illinois Natural History Survey, Champaign, Illinois.

Tompsett, D. H. 1939. *Sepia. Liverpool Marine Biology Committee, Memoirs on Typical British Marine Plants & Animals volume 32.* The University Press of Liverpool. 184 pp.

Toll, R. B. 1982. *The Comparative Morphology of the Gladius in the Order Teuthoidea (Mollusca: Cephalopoda) in Relation to Systematics and Phylogeny.* Doctoral Dissertation, University of Miami, Coral Gables, Florida. 390 pp.

Villanueva, R. 1992. Continuous spawning in the cirrate octopods *Opisthoteuthis agassizii* and *O. vossi*: features of sexual maturation defining a reproductive strategy in cephalopods. *Marine Biology* 114:265-275.

Voss, G. L. 1977. Appendix II. Classification of Recent cephalopods. *Symposia of the Zoological Society of London* 38: 575-579.

Voss, G. L. 1988. Evolution and phylogenetic relationships of deep-sea octopods (Cirrata and Incirrata) *In: The Mollusca, 12: Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 253-276. Academic Press, New York.

Voss, N. A. and R. S. Voss. 1983. Phylogenetic relationships in the cephalopod family Cranchiidae (Oegopsida). *Malacologia* 23(2):397-426.

Ward, D. V. and S. A. Wainwright. 1972. Locomotory aspects of squid mantle structure. *Journal of Zoology, London* 167: 437-449.

Young, J. Z. 1971. *The Anatomy of the Nervous System of* Octopus vulgaris. Clarendon Press, Oxford. 690 pp.

Young, J. Z. 1977. Brain, behaviour and evolution of cephalopods. *Symposia of the Zoological Society of London* 38:377-434.

Young, J. Z. 1988. Evolution of the cephalopod brain. *In: The Mollusca, 12: Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 215-228. Academic Press, New York.

Young, R. E. 1962. *The Anatomy of the Vampire Squid.* Master's Thesis, University of Southern California, Los Angeles. 234 pp.

Young, R. E. 1967. Homology of retractile filaments of vampire squid. *Science* 156(3782):1633-1634.

Young, R.E. and R. Harman. In press. The phylogeny of the enoploteuthid families. *Smithsonian Contributions to Zoology.*

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Preliminary cladistic analyses of relationships among loliginid squids (Cephalopoda: Myopsida) based on morphological data

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Abstract: Most species in the cephalopod taxon Loliginidae have a near-shore habitat and are commercially important, yet phylogenetic relationships within the group have not been examined. In this study, relationships among loliginid species are analyzed using cladistic methods. Forty-eight morphological characters for 48 species (40 loliginid species and eight outgroup taxa) were collected through examination of museum specimens and primary literature and coded into a matrix for cladistic analysis. Both unweighted and successive weighting maximum-parsimony analyses were undertaken, and the phylogenetic signal of the data was evaluated. Unweighted analyses support the hypothesis of monophyly for Loliginidae, and suggest some well-supported sister species and crown-clade relationships (such as *Alloteuthis* Wülker and *Sepioteuthis* Blainville), but the positions of these groups relative to one another cannot be resolved due to the large number of most-parsimonious trees. Successive weighting analyses showed support for some additional major clades (*Photololigo* Natsukari and *Nipponololigo* Natsukari), and provided insight into the cladistic information value of the characters in the analysis. Continued collection of morphological and internal anatomical data for these species for all stages of the life cycle, as well as the addition of molecular data to the analysis, could help resolve relationships within the group.

The cephalopod taxon Loliginidae contains over 40 species of neritic squids found on most tropical and temperate continental margins around the world (Roper *et al.*, 1984). In many regions, these squids are found in high abundance near shore, particularly when spawning, and some species form integral links in coastal marine ecosystems (such as *Loligo opalescens* Berry, 1911, in Monterey Bay, California; see Morejohn *et al.*, 1978). Many loliginid species are commercially harvested (Roper *et al.*, 1984). In addition, the giant axons of certain loliginids (such as *L. pealei*, *L. vulgaris*, and *L. opalescens*) have served as important model systems in neurophysiological research (*e. g.* Young, 1938; Gilbert *et al.*, 1990; Rosenthal and Gilly, 1993).

Despite the ecological, economic, and scientific importance of loliginid squids, their phylogeny remains unresolved, and their taxonomy is confused (Voss, 1977). Numerous recent works (Natsukari, 1983, 1984; Brakoniecki, 1986; Alexeyev, 1992; Vecchione *et al.*, in press) have sought to clarify loliginid taxonomy using key morphological characters. Brakoniecki (1986), who examined loliginid hectocotylus morphology, grouped the species he studied into six hectocotylus types. He based a new generic-level classification on his findings, and proposed an evolutionary zoogeographic scenario for the radiation of the group. Vecchione *et al.* (in press) have used a phenetic analysis of loliginids as the basis of a generic-level taxonomy. Others, including Augustyn and Grant (1988) and Brierley and Thorpe (1994), have used allozyme electrophoresis to address problems of loliginid relationships. Despite such efforts, these authors admit that further work is necessary to clarify loliginid phylogeny.

No researchers have explicitly addressed loliginid phylogenetic relationships using cladistic methodology. Cladistic analysis allows many discrete character data to be considered simultaneously using an explicit, simple optimality criterion in which the preferred tree is the one which requires minimal assumptions of convergence and reversal across all characters in the analysis (the criterion of maximum parsimony) (Edwards and Cavalli-Sforza, 1964; Camin and Sokal, 1965; Kluge and Farris, 1969; Fitch, 1971; Farris, 1983; Sober, 1988; see Wiley *et al.*, 1991, for a review of cladistic philosophy and techniques). Cladistic analysis yields a hypothesis of phylogenetic relationships with which to interpret biogeographical patterns and character evolution (Brooks and McClennan, 1991; Maddison and Maddison, 1992). Phylogenetic hypotheses can also be used to construct taxonomic schemes (de Queiroz and Gauthier, 1990, 1992).

For this study, many aspects of loliginid morphology (particularly the hectocotylus, arm and tentacle-club sucker rings, spermatophores, fins, and some aspects of internal anatomy) were coded into a matrix for cladistic analysis to examine species-level relationships within the family. These data were analyzed using a maximum-parsimony algorithm program, and the results were compared with traditional taxonomic schemes and recent reclassifications. The strengths and problems of using morphological characteristics for examining loliginid evolution are addressed, and topics for future research are briefly outlined.

MATERIALS AND METHODS

DATA COLLECTION

Forty-eight morphological characters for 40 species of loliginid squids and eight outgroup taxa were coded into a data matrix in MacClade 3.04 (Maddison and Maddison, 1992) (Appendix I). Character states were determined through direct study of museum specimens at the National Museum of Natural History, the California Academy of Sciences, and the Invertebrate Museum at the University of Miami Rosenstiel School of Marine and Atmospheric Science, as well as published species descriptions (Appendix III). Some described loliginid species [including *Loligo arabica* (Ehrenberg, 1831) and newly described species such as *Photololigo robsoni* (Alexeyev, 1992)] were not included in this study because descriptions of these species were insufficient to code many characters with confidence, and specimens were not available for examination at the institutions I visited.

The outgroup method (Watrous and Wheeler, 1981; Maddison *et al.*, 1984) was used to polarize character transformations in this study. The eight outgroup taxa represent a broad range of decapod cephalopod diversity. Four oegopsid taxa (*Moroteuthis* Verrill, *Todarodes* Steenstrup, *Ctenopteryx* Appellöf, and *Bathyteuthis* Hoyle), two sepiolid taxa (*Euprymna* Steenstrup and *Rossia* Owen), and one sepiid taxon (*Sepia* Linné) were included, along with *Pickfordiateuthis pulchella* Voss, 1953. *Pickfordiateuthis* was originally described by Voss (1953) as a monospecific taxon closely related to Loliginidae as another member of the Myopsida. Recently, two new species of *Pickfordiateuthis* have been described, and *Pickfordiateuthis* has been subsumed within Loliginidae (Brakoniecki, 1996). For this analysis, *P. pulchella* was used as a representative of this group of squids. These outgroups were selected for a variety of reasons. In some cases, earlier authors have suggested that certain oegopsid taxa are close relatives of Loliginidae (*e. g. Ctenopteryx*; Young, 1991). In contrast, Berthold and Engeser (1987) suggested that Loliginidae, Sepiolidae, and Sepiidae are all closely related members of the Myopsida. Morphological similarities also exist between loliginids and various active nektonic oegopsids like *Todarodes*. Due to this uncertainty, a diversity of cephalopod taxa were included as outgroups in this study.

Many characters included in this analysis (such as hectocotylus morphology, sucker ring dentition, and fin shape) have been used in traditional studies of loliginid taxonomy, but have never been objectively analyzed simultaneously. In certain cases, some of these characters have been presumed to be informative at some taxonomic levels but not at others. For example, arm-sucker ring dentition generally has been used to distinguish between very similar species (Natsukari, 1983; Brakoniecki, 1986), but it has not been used as a taxonomic character above this level. In other cases, some characters have been examined only in those supraspecific taxa where they help unite or separate species (*e. g.* number of trabeculae per marginal club sucker in *Alloteuthis* Wülker; Hanlon *et al.*, 1992), and might not have been thoroughly examined in all loliginid species. Still other characters (*e. g.* spermatophore morphology) have been examined widely in loliginids, but have not figured importantly so far in cephalopod systematic studies (Hess, 1987; deMaintenon, 1990). Some characters were found to vary among loliginid species but were consistent within species, and were included in this analysis. Certain characters of traditional importance were avoided because they appeared to vary within certain species, and too few specimens were available to resolve these inconsistencies. For example, thickenings of the lateral edges of the vane of the gladius are for some authors important diagnostic characters for the genera *Doryteuthis* Naef and *Loligo* Lamarck but have been found to be variable within species (Cohen, 1976; Toll, 1982). Despite this, some polymorphic characters (characters that vary within some terminal taxa) were included in the analysis. Characters exhibiting intraspecific variation can contain strong phylogenetic signal (although generally not as strong as fixed characters) and thus should not be ignored or simply coded as fixed in cladistic analyses (Wiens, 1995). *A priori* assumptions about the information value of characters (other than inclusion of "traditional" well-studied characters in the analysis) were avoided, but inevitably (as in all phylogenetic studies) some characters that could be phylogenetically informative have been excluded. Appendix II lists the characters, argumentation and coding scheme used in this analysis.

Data for some characters are either not applicable for certain taxa ("n" in Appendix I) or could not be determined ("?" in Appendix I). Inapplicable characters usually refer to some aspect of a structure which is not present in all taxa in the analysis (*e. g.* "hectocotylus dorsal row sucker morphology" in taxa which do not possess hectocotyluses). In some cases, coding of "inapplicable" characters in this manner can cause problems in cladistic analyses (Maddison, 1993), but the solution advocated by Maddison

(1993) – combining all such characters into one character with many states – is often impractical and can lead to the loss of phylogenetic information. For example, in the case of this analysis, fusing all nine hectocotylus characters into one multistate character with many states was not performed. Fusing all hectocotylus characters into a single character with many distinct unordered states does not allow homology statements for individual aspects of hectocotylus morphology. It is possible, for instance, that species that possess a particular type of modified sucker, irrespective of the region of the arm that bears the modification, constitute a monophyletic group relative to species with other types of sucker modification, or vice versa. If the hectocotylus characters were fused into one unordered multistate character, this information would be lost – only species with almost exactly the same hectocotylus morphology (*i. e.* the same coded state for the single hectocotylus character) would be grouped together. Maddison's (1993) method is valid, but maximally conservative, and a great deal of phylogenetic information could be lost by collapsing characters in this way.

DATA ANALYSES

These data were analyzed using the maximum parsimony program PAUP 3.1.1 (Swofford, 1993). When terminal taxa were coded as having multiple states for one or more characters, these characters were interpreted as "polymorphic." All characters were unordered binary or multistate characters, and were weighted equally for all initial analyses. The use of equal weighting does not mean that each character in the matrix is of equal informative value. I have chosen to use equal weighting simply because I have no compelling reason to use any particular *a priori* differential weighting scheme (see discussion in Eernisse *et al.*, 1992). Heuristic searches were performed with 100 replications of random stepwise addition of taxa using tree bisection-reconnection swapping with one tree held at each step. The maximum number of trees stored for each search (MAXTREES) was 10,000. The COLLAPSE option was turned off for some analyses in an effort to find all regions of "islands" of most-parsimonious trees (Maddison, 1991; Swofford, 1993). Following Maddison's (1991) suggestion, a total of ten heuristic analyses were done to search for other islands and to examine the level of support for various branches within cladograms. A strict consensus cladogram was computed from the trees from each of the ten heuristic searches, for a total of ten strict consensus cladograms. A strict consensus of these ten strict consensus trees (a "grand strict" consensus cladogram) collapsed all ambiguities and revealed elements common to all trees from all ten searches.

After these preliminary analyses, two methods were used in an attempt to reduce tree number and investigate the phylogenetic utility of individual characters. The "reweight characters" option in PAUP was used to successively weight characters after each heuristic search (following the approach of Farris, 1969). Farris (1989) proposed the rescaled consistency index (RCI) and suggested its use in successive weighting analyses. In this analysis, characters were reweighted on the basis of their best rescaled consistency index value across the trees from the previous search. A heuristic search (following the same parameters as described above) was performed using the reweighted characters. Rounds of successive weighting were repeated until overall strict consensus tree topology did not change from one round to the next, or until character weights did not change after reweighting. As in preliminary analyses, strict consensus cladograms from the final round of weighting were combined, and a grand strict consensus cladogram was computed. This allowed common elements found in all successive weighting analyses to be determined.

A recently described technique called "safe taxonomic reduction" (Wilkinson, 1995) was also used in an effort to reduce the number of trees found. Analysis of matrices containing taxa with many missing data can result in an inordinately large number of equally parsimonious trees, because taxa with a large percentage of missing data (termed negatively underdetermined taxa) can occupy a number of equally parsimonious positions (Wilkinson, 1995). Consensus methods can be used to find common elements across multiple most-parsimonious trees (MPT's), but negatively underdetermined taxa can obfuscate patterns of relationship among other taxa that are found in all trees, yielding an extreme lack of resolution in strict consensus cladograms. The goal of safe taxonomic reduction is to remove negatively underdetermined taxa from the analysis without losing information about relationships (*i. e.* without altering patterns of relationships among the remaining taxa). This reduction in the number of negatively underdetermined taxa often reduces greatly the number of MPT's found by parsimony analysis. Increased resolution in consensus cladograms is often found after safe taxonomic reduction. In these analyses, the only taxa that could be safely removed from the ingroup using Wilkinson's technique were *Alloteuthis africana* and *A. media*, which were taxonomic equivalents of *A. subulata*, and *Loliolus affinis*, which was a taxonomic equivalent of *L. hardwickei*.

The phylogenetic signal of the data was evaluated using the g_1 test of Hillis and Huelsenbeck (1992). Based on simulation data, Hillis and Huelsenbeck (1992) proposed the use of the g_1 statistic (a measure of skewness) as one way to evaluate the ratio of signal to random noise in phylogenetic data. They found that high degrees of left-skew in plots of random tree distributions or total tree distributions obtained through exhaustive searches correlated well with the success of parsimony methods in finding the true phylogeny in simulation studies. Ten thousand random trees were generated based on this matrix using PAUP 3.1.1 with multistate taxa interpreted as polymorphic. The g_1 from this random tree distribution was compared to 95% and 99% confidence-limit values obtained from simulations with 50 binary or multistate characters and 25 taxa performed by Hillis and Huelsenbeck (1992), which should provide an approximate conservative comparison. In addition, all clades or sister-species groupings found across all trees (*i.e.* groupings retained in the grand strict consensus) were constrained to examine if phylogenetic signal was clustered within these groups. If the g_1 value (a negative value in left-skewed distributions) increases greatly after these constraints are applied, a large proportion of the phylogenetic signal in the matrix can be clumped within the universally supported clades, and is not evenly distributed across all data (Hillis and Huelsenbeck, 1992).

RESULTS

All unconstrained, unweighted analyses of all characters and all taxa resulted in 10,000+ most-parsimonious trees — the MAXTREES limit of 10,000 was reached in all analyses. The strict consensus cladogram of all sets of 10,000 trees from all ten unconstrained and unweighted heuristic searches is shown in Fig. 1. Tree lengths and statistics of the constituent trees are shown in Table 1.

The strict consensus cladogram of the ten final strict consensus trees from successive weighting was marginally more resolved than the strict consensus of the unweighted analyses (Fig. 2). Characters that were maximally and minimally weighted after multiple rounds of successive weighting are shown in Table 2.

Employing safe taxonomic reduction and removing *Alloteuthis africana, A. media,* and *Loliolus affinis* from the analyses had no apparent effect on the number of trees found in either unweighted or successive weighting analyses, or in the topology of the strict consensus cladograms from these analyses. Ten thousand trees were still found in all analyses following the removal of these taxa. The relative positions of *A. subulata* and *L. hardwickei* alone were the same as their positions when all taxa were included in the analysis.

Table 1. Tree statistics for 10,000 most-parsimonious trees found in the ten unweighted heuristic analyses.

Indices for unweighted trees
Consistency index (CI) = 0.582
Homoplasy index (HI) = 0.572,
Retention index (RI) = 0.663
Rescaled consistency index (RCI) = 0.386
Treelength (TL) = 194

Table 2. Maximally and minimally weighted characters across all weighted analyses (based on best rescaled consistency index fit). Characters that vary within the ingroup are denoted by an asterisk (*).

Maximally weighted characters Character Number	Description
1	rachidian cusps of radula
2	lateral cusps of radula
5	number of arm sucker rows
14	retractile tentacles
15	trabeculae number*
20	photophores on ink sac*
25	ventral row modification*
29	fused crest in ventral row of hectocotylus*
38	spermatophore placement*
40	spiral filament
43	pores on ink sac*
46	muscular septum
47	nuchal cartilage
48	digestive gland
Minimally weighted characters (weighted to zero) Character Number	**Description**
4	arm sucker rings
10	central manus sucker teeth*
13	marginal manus sucker teeth*
16	buccal membrane lobes
18	buccal lappet sucker teeth
19	buccal membrane formula
27	length of hectocotylus*

The g_1 skewness test of Hillis and Huelsenbeck (1992) suggests the presence of significant phylogenetic signal in the unweighted data matrix. The g_1 value for 100,000 random trees derived from this data matrix was -1.275647. This value was appreciably below the critical values for 50 binary or 50 four-state characters for 25 taxa [95% confidence limit for binary characters = -0.10, for four-state characters = -0.12; 99% confidence limit for binary characters = -0.11, for four-state characters = -0.13, based on simulation studies (Hillis and Huelsenbeck, 1992)]. When this procedure was repeated with the universally supported clades retained (using the grand strict consensus as a constraint), the g_1 value for a distribution of 100,000 random trees was -0.502625. This value suggests that even when universally supported clades are constrained, significant phylogenetic signal remains.

DISCUSSION

A small number of ingroup sister-species and subclade relationships were supported in the multiple

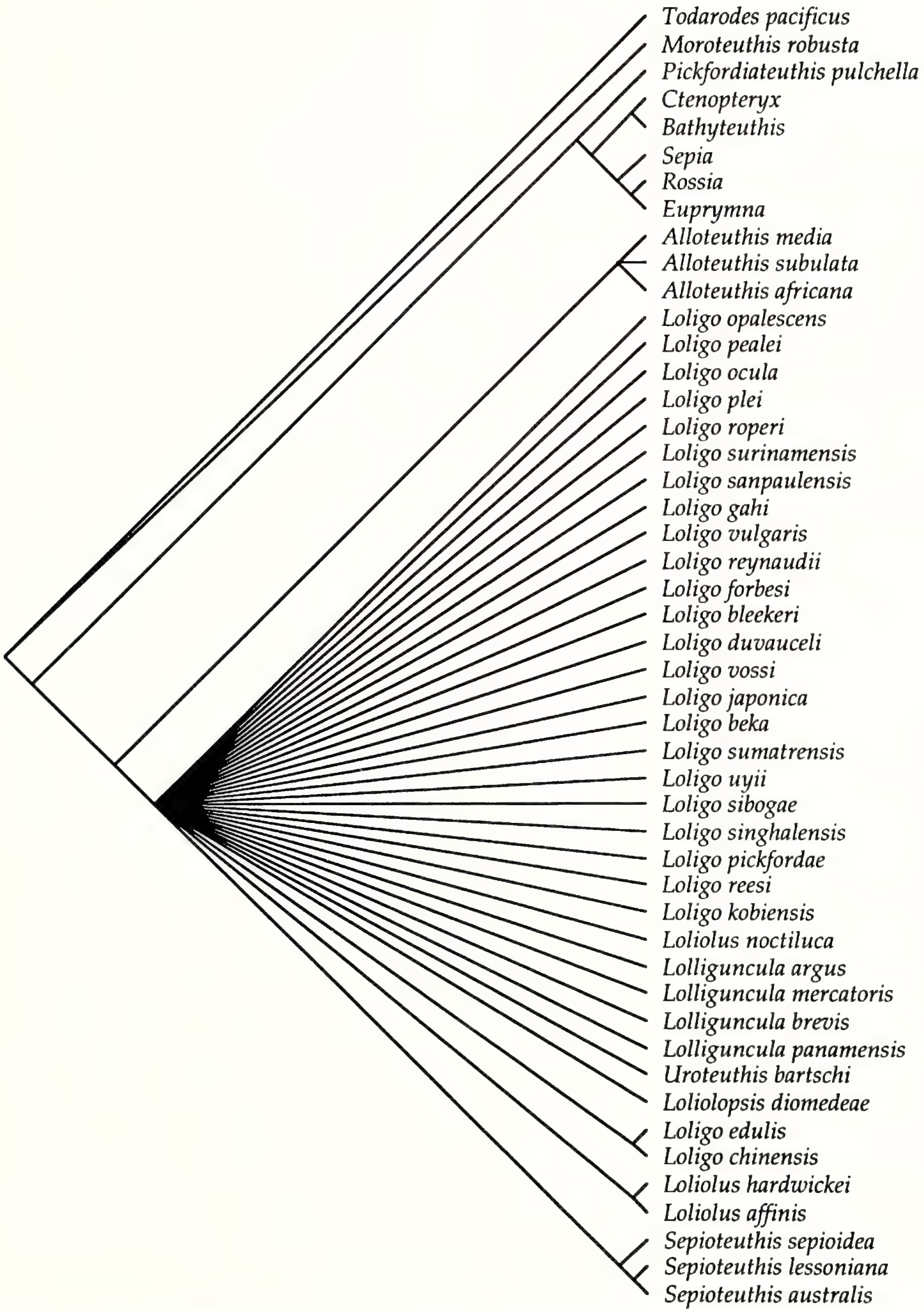
Todarodes pacificus
Moroteuthis robusta
Pickfordiateuthis pulchella
Ctenopteryx
Bathyteuthis
Sepia
Rossia
Euprymna
Alloteuthis media
Alloteuthis subulata
Alloteuthis africana
Loligo opalescens
Loligo pealei
Loligo ocula
Loligo plei
Loligo roperi
Loligo surinamensis
Loligo sanpaulensis
Loligo gahi
Loligo vulgaris
Loligo reynaudii
Loligo forbesi
Loligo bleekeri
Loligo duvauceli
Loligo vossi
Loligo japonica
Loligo beka
Loligo sumatrensis
Loligo uyii
Loligo sibogae
Loligo singhalensis
Loligo pickfordae
Loligo reesi
Loligo kobiensis
Loliolus noctiluca
Lolliguncula argus
Lolliguncula mercatoris
Lolliguncula brevis
Lolliguncula panamensis
Uroteuthis bartschi
Loliolopsis diomedeae
Loligo edulis
Loligo chinensis
Loliolus hardwickei
Loliolus affinis
Sepioteuthis sepioidea
Sepioteuthis lessoniana
Sepioteuthis australis

Fig. 1. Grand strict consensus cladogram of ten strict consensus cladograms derived from ten heuristic searches yielding 10,000 trees each. Tree statistics for the trees upon which this strict consensus is based are shown in Table 1.

unweighted heuristic analyses (Fig. 1), but deeper branching orders and relationships among loliginid squids remain unresolved, despite the relatively high phylogenetic signal suggested by the g_1 test. Successive weighting analyses consistently suggested support for some higher-level relationships (Fig. 2), but even with successive weighting and safe taxonomic reduction, tens of thousands of most-parsimonious trees were found, and, despite some interesting findings, the resulting grand strict consensus cladograms are largely unresolved.

Monophyly of Loliginidae was supported by all unweighted and weighted analyses. Myopsida (traditionally comprised of Loliginidae + Pickfordiateuthidae) was found to be paraphyletic in all unweighted and weighted analyses. Brakoniecki's (1996) inclusion of *Pickfordiateuthis* within Loliginidae is not justified based on these analyses. Within the ingroup, only four clades were consistently supported in all unweighted analyses, and two of these are sister-species groupings. Monophyly of the genus *Alloteuthis*, consisting of three species of small, slender squids found in the eastern Atlantic along the coasts of Europe and Africa (*A. africana, A. media,* and *A. subulata*) was supported in all analyses, and was the sister taxon to the rest of Loliginidae. *Alloteuthis* appears to have diverged from the other loliginid species early in the history of the group. Monophyly of the genus *Sepioteuthis* Blainville, which is comprised of *S. australis* and *S. lessoniana* (both Indo-West Pacific species), and *S. sepioidea* (a Caribbean species) was also supported in all unweighted analyses. Two synapomorphies appear to unite the three species of *Sepioteuthis* – a longitudinally oval fin shape uniquely derived within Loliginidae, and the large size of the dorsal row of papillae relative to the ventral row in the modified portion of the hectocotylus. In addition to these three-taxon clades, two sister-species pairings were consistently found – *Loligo chinensis* and *L. edulis* in one pairing, *Loliolus hardwickei* and *L. affinis* in the other. All other relationships within Loliginidae are unresolved in the unweighted analyses.

There are a number of possible explanations for this lack of resolution. First, the number of characters employed (48) is relatively low compared to the number of terminal taxa (48) included in the analysis. Another important factor might be that many of the characters used in the analysis are highly homoplastic, showing evidence of multiple convergences or reversals throughout the evolution of this group. Several of the characters included in this analysis have been used extensively in decapod cephalopod taxonomic studies, and appear to be very useful for distinguishing species, but when relationships among all loliginid species are studied and characters are atomized for cladistic analysis, individual characters can exhibit high levels of homoplasy.

Successive weighting techniques have been used by many authors to reduce the number of most-parsimonious trees, to increase resolution in consensus trees, as a heuristic tool to investigate the cladistic informativeness of characters, and to study the effect of homoplastic characters in cladistic analyses (Farris, 1969; Carpenter, 1988, 1994), but some authors have criticized the use of successive weighting techniques (Swofford and Olsen, 1990) or have urged caution in the interpretation of results from successive weighting analyses (Maddison and Maddison, 1992; Swofford, 1993; Suter, 1994). Some investigators (*e. g.* Suter, 1994) have found that the parameters used in successive weighting analyses can have an effect on the outcome of successive weighting analyses. In addition, successive weighting does not necessarily reduce the number of most-parsimonious trees – in this analysis, the tree buffer limit was reached on all analyses, unweighted or weighted. Successive weighting can reduce the weight of highly homoplastic characters to zero, effectively removing them from the analysis [seven characters were weighted to zero by the final round of weighting in these analyses (Table 2)]. As the number of characters actually included in the analysis drops, resolution in strict consensus cladograms likely will drop, particularly when the number of characters is small relative to the number of taxa in the analysis.

Farris (1969) and Carpenter (1988, 1994) have strongly supported successive weighting as simply an extension of the concept of cladistic reliability, or the degree of fit between a character and the phylogeny (Farris, 1969). Successive weighting allows *a posteriori* weighting based on the cladistic information value of the characters in the matrix. Characters that show little homoplasy when evaluated in conjunction with all other characters in the matrix are increased in weight (or set at a maximum base weight in PAUP 3.1.1) relative to characters exhibiting more homoplasy in subsequent rounds of analysis, while highly homoplastic characters are reduced in relative weight. Carpenter (1988, 1994) has argued that successive weighting "allows the characters of a given data set to judge themselves in terms of their reliability; that is, best fit to the solution supported by all the characters" (Carpenter, 1994: 216).

Successive weighting analyses supported all clades found in earlier, unweighted analyses, and suggested three other groupings not found in unweighted analyses. Two major clades were supported in all final weighted analyses – a clade consisting of all loliginid species possessing paired bioluminescent organs on the ink sac [*Loligo edulis, L. chinensis, L. duvauceli, L. sibogae, L. singhalensis, L. pickfordae* (Adam, 1954), *L. reesi, Loliolus noctiluca,* and *Uroteuthis bartschi*, all found in the Indo-West Pacific], and a clade consisting of seven other Indo-West Pacific species (*Loligo beka, L. japonica, L. sumatrensis, L. uyii, L. kobiensis, Loliolus hardwickei,* and *L. affinis*). In addition,

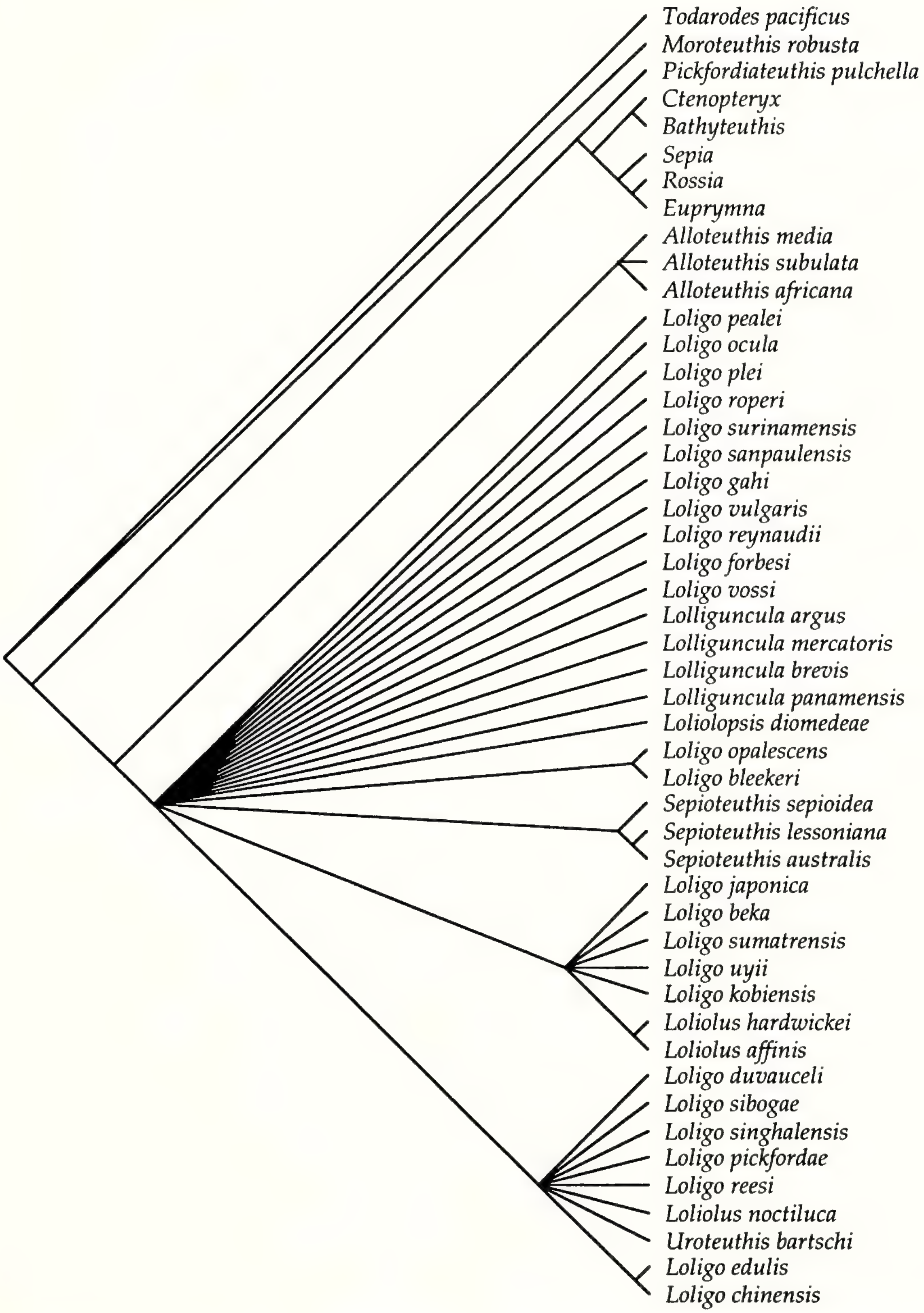
Todarodes pacificus
Moroteuthis robusta
Pickfordiateuthis pulchella
Ctenopteryx
Bathyteuthis
Sepia
Rossia
Euprymna
Alloteuthis media
Alloteuthis subulata
Alloteuthis africana
Loligo pealei
Loligo ocula
Loligo plei
Loligo roperi
Loligo surinamensis
Loligo sanpaulensis
Loligo gahi
Loligo vulgaris
Loligo reynaudii
Loligo forbesi
Loligo vossi
Lolliguncula argus
Lolliguncula mercatoris
Lolliguncula brevis
Lolliguncula panamensis
Loliolopsis diomedeae
Loligo opalescens
Loligo bleekeri
Sepioteuthis sepioidea
Sepioteuthis lessoniana
Sepioteuthis australis
Loligo japonica
Loligo beka
Loligo sumatrensis
Loligo uyii
Loligo kobiensis
Loliolus hardwickei
Loliolus affinis
Loligo duvauceli
Loligo sibogae
Loligo singhalensis
Loligo pickfordae
Loligo reesi
Loliolus noctiluca
Uroteuthis bartschi
Loligo edulis
Loligo chinensis

Fig. 2. Grand strict consensus cladogram of ten strict consensus cladograms from successive weighting analyses from each of ten heuristic searches.

Loligo opalescens and *L. bleekeri* were found to be sister species in all final weighted analyses.

The major putative synapomorphy uniting the bioluminescent loliginids are the paired bean-shaped bacterial photophores (luminescent organs) on the ventral side of the ink sac. This clade is similar to the proposed genus *Photololigo* Natsukari (1984), which includes five of these species (*Loligo edulis, L. duvauceli, L. chinensis, L. singhalensis,* and *L. sibogae*) as well as *L. arabica*, which was not included in this analysis. Natsukari's *Photololigo*, however, does not include *L. reesi, L. pickfordae, Loliolus noctiluca,* or *Uroteuthis bartschi*. Results of this weighted analysis support Vecchione *et al.*'s (in press) *Photololigo* as a monophyletic group. However, these authors divide *Photololigo* into two smaller groups – a subgenus *Photololigo* and a subgenus *Uroteuthis* (consisting only of one species – *U. bartschi*). It is possible that *U. bartschi* is a derived member of the photololiginid clade. If this is true, Vecchione *et al.*'s (in press) subgenus *Photololigo* is paraphyletic with respect to their subgenus *Uroteuthis*. Unfortunately, cladistic analysis of these data cannot address this question. Alexeyev (1992) has reported that some specimens of *Lolliguncula mercatoris* and a single specimen of *Loligo forbesi* appeared to possess photophores on the ink sac. In this analysis, these species are considered to lack photophores, pending further investigation (as suggested by Vecchione *et al.*, in press). If Alexeyev's (1992) findings are accurate, they must be accounted for in future phylogenetic studies of this group.

More detailed studies of the primary synapomorphy that unites the species of *Photololigo* – the photophores themselves – might help resolve these problems. In *Euprymna*, the bioluminescent organ is the product of a complex interaction between the squid and symbiotic luminescent bacteria (McFall and Ruby, 1991). Further investigations of this interaction and its effects on photophore morphology in all photololiginid squids (*e. g.* Haneda, 1963; Pringennies and Jørgensen, 1994) could illuminate species relationships within the clade.

The other major clade found in the weighted analyses is very similar to Natsukari's (1983) *Nipponololigo*, a proposed subgenus of *Loligo* comprised of *L. japonica, L. uyii, L. kobiensis,* and *L. beka.* The successive weighting analyses support the inclusion of *L. sumatrensis* and the *Loliolus affinis-hardwickei* sister-species grouping within a broader *Nipponololigo* clade. The two synapomorphies uniting these species are the sucker morphology of the dorsal and ventral rows of the hectocotylus. The pedicels of the dorsal row suckers are fused with their protective membrane and widened into fleshly slabs (Natsukari, 1983; Brakoniecki, 1986). In most of these species, the slabs retain small suckers; in *L. affinis* and *L. hardwickei*, however, the suckers are not present on the tops of the slabs. In these analyses, the lack of suckers on the tops of the slabs was revealed as a synapomorphy uniting these two species as sister taxa. In the ventral row of the hectocotylus, all species in the *Nipponololigo* clade possess minute, apparently suckerless papillae. Vecchione *et al.* (in press) have proposed the name *Loliolus* to include all members of Natsukari's *Nipponololigo* as well as *L. affinis* and *L. hardwickei.* These species are divided into two subgenera – *Loliolus (Loliolus)* (comprised of *L. affinis* and *L. hardwickei*) and *Loliolus (Nipponololigo)* (comprised of the species in Natsukari's *Nipponololigo*, plus *L. sumatrensis*). As with *Photololigo,* these analyses generally support their conclusion, although a paraphyletic *Nipponololigo* (with respect to *L. affinis* and *L. hardwickei*) is a possibility that cannot be addressed with these data alone.

Loligo opalescens and *L. bleekeri* constitute a sister-species pairing in all weighted analyses. Brakoniecki (1986) anticipated this result. He proposed that the epithet *Doryteuthis* (subgenus *Doryteuthis*) be applied to six species of loliginid squids. Five of these species (*Loligo plei, L. roperi, L. sanpaulensis, L. gahi,* and *L. opalescens*) are found in American waters, while one species (*L. bleekeri*) is found only in Japanese waters. *Doryteuthis (Doryteuthis)* and *Sepioteuthis* are the only geographically disjunct groupings described by Brakoniecki (1986). Brakoniecki proposed a causal explanation for the distribution of *Doryteuthis (Doryteuthis)* – he suggested that a slight rise in water temperature in the northern Pacific Ocean could have allowed the *L. bleekeri-opalescens* common ancestor to disperse from the eastern Pacific coast of North America to Japan via the Aleutians. The results of the weighted analyses support a sister-species relationship between *L. bleekeri* and *L. opalescens*, but, due to the lack of resolution of relationships among other *Doryteuthis (Doryteuthis)* species, a monophyletic subgenus *Doryteuthis* (*sensu* Brakoniecki, 1986) remains a possibility, but is not directly supported. Due to the overall lack of resolution, the possible ancestral range of the *L. bleekeri-opalescens* ancestor cannot be examined.

In addition to these putative clades, certain species presently grouped in the genus *Lolliguncula* (*L. panamensis, L. mercatoris,* and *L. brevis*), together with *Loliolopsis diomedeae*, were found in all strict consensus trees from all weighted analyses. However, the position of *Lolliguncula argus* was variable across these trees. In some consensus trees, *L. argus* was completely outside the clade comprised of the rest of the *Lolliguncula* species plus *Loliolopsis diomedeae*. In other trees, *L. argus* was found to be a highly derived member of the *Lolliguncula* + *Loliolopsis* clade. Due to the variable position of this taxon, the *Lolliguncula* + *Loliolopsis* clade collapsed in the overall strict consensus cladogram (Fig. 2).

Successive weighting analyses can provide heuristic insight into the information value of the characters in the analysis. Most informative, consistent characters found after multiple rounds of successive weighting are invariant within the ingroup (Table 2). Few characters that vary within the ingroup appear to have high rescaled consistency indices across all initial unweighted trees. Also, several characters show varying amounts of homoplasy across initial unweighted most-parsimonious trees, and subsequently have low weights after successive weighting analyses.

The overall lack of resolution in strict consensus trees found after these unweighted and weighted analyses and the low weights of many characters after successive weighting highlight the limited utility of using only gross external morphological characters to investigate loliginid squid phylogeny. Despite this general conclusion, external morphological characters should not be ignored in future investigations of loliginid relationships. The Hillis and Huelsenbeck (1992) g_1 test shows significant phylogenetic structure in the data matrix. Some of the characters used in this analysis do appear to carry appreciable phylogenetic information. Undoubtedly, more data must be gathered to test the results of these analyses and to resolve relationships among these squids. Little is known about comparative internal anatomy in loliginid squids, although excellent studies have been done of particular species (*e.g.* Williams, 1909). For example, the anatomy of the nervous system and circulatory system, and perhaps aspects of juvenile development, are particularly promising systems for inclusion in cladistic analysis. Many neurophysiological studies have been performed on a broad range of loliginid squids, including *Loligo opalescens, L. pealei, L. vulgaris, Sepioteuthis lessoniana, Lolliguncula brevis,* and *Alloteuthis media* (*e.g.* Brown *et al.*, 1991; Chrachri and Williamson, 1993; Fishman and Metuzals, 1993; Preuss and Budelmann, 1995). Due to the ease of culturing some of these species in the laboratory (Lee *et al.*, 1994), and the giant axons of many loliginids, more comparative neurological studies undoubtedly will be performed. These data could be combined with other morphological and anatomical data for cladistic analysis.

In addition to internal anatomical information, molecular data could aid in resolving loliginid relationships. Recently, Yeatman and Benzie (1994) have found genetic evidence of cryptic speciation within *Photololigo edulis* and *P. chinensis*, providing evidence of the power of molecular techniques in species-level research of loliginid squids. An ongoing sequencing study of two mitochondrial genes (the 16S ribosomal DNA gene and the cytochrome *c* oxidase subunit I gene) could shed light on loliginid relationships (Anderson, unpub.). Relationships among loliginid squids at the species level and investigations of cladogenesis and biogeography within this group will be possible only through an examination of multiple sources of data, including morphological, anatomical, and molecular sequence data.

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LITERATURE CITED

Adam, W. 1950. Notes sur les cephalopodes, XXII. Deux nouvelles espèces de la côte africaine occidentale. *Bulletin du Institut Royal des Sciences Naturelles de Belgique* 26:1-9.

Adam, W. 1954. Cephalopoda, IV. Céphalopodes à l'exclusion des genres *Sepia, Sepiella* et *Sepioteuthis. Siboga-Expeditie* 55c:123-198.

Alexeyev, D. O. 1992. The systematic position of bioluminescent squids of family Loliginidae (Cephalopoda, Myopsida). *Zoologicheskii Zhurnal* 71:12-23.

Augustyn, C. J. and W. S. Grant. 1988. Biochemical and morphological systematics of *Loligo vulgaris vulgaris* Lamarck and *Loligo vulgaris reynaudi* d'Orbigny nov. comb. (Cephalopoda: Myopsida). *Malacologia* 29:215-233.

Berry, S. S. 1911. A note on the genus *Lolliguncula. Proceedings of the Academy of Natural Sciences of Philadelphia* 1911:100-105.

Berry, S. S. 1929. *Loliolopsis chiroctes*, a new genus and species of squid from the Gulf of California. *Transactions of the San Diego Society of Natural History* 5:263-282.

Berthold, T. and T. Engeser. 1987. Phylogenetic analysis and systematization of the Cephalopoda (Mollusca). *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* 29:187-220.

Brakoniecki, T. F. 1980. *Lolliguncula tydeus*, a new species of squid (Cephalopoda: Myopsida) from the Pacific coast of Central America. *Bulletin of Marine Science* 30:424-430.

Brakoniecki, T. F. 1984. A full description of *Loligo sanpaulensis* Brakoniecki, 1984 and a redescription of *Loligo gahi* d'Orbigny, 1835, two species from the southwest Atlantic. *Bulletin of Marine Science* 34:435-448.

Brakoniecki, T. F. 1986. *A Generic Revision of the Family Loliginidae (Cephalopoda: Myopsida) Based Primarily on the Comparative Morphology of the Hectocotylus.* Doctoral Dissertation, University of Miami, Coral Gables, Florida. 164 pp.

Brakoniecki, T. F. 1996. A revision of the genus *Pickfordiateuthis* Voss, 1953 (Cephalopoda: Myopsida). *Bulletin of Marine Science* 58:9-28.

Brakoniecki, T. F. and C. F. E. Roper. 1985. *Lolliguncula argus*, a new species of loliginid squid from the tropical eastern Pacific.

Proceedings of the Biological Society of Washington 98:47-53.

Brierley, A. S. and J. P. Thorpe. 1994. Biochemical genetic evidence supporting the taxonomic separation of *Loligo gahi* from the genus *Loligo. Antarctic Science* 6:143-148.

Brooks, D. R. and D. A. McClennan. 1991. *Phylogeny, Ecology and Behavior: a Research Program in Comparative Biology.* University of Chicago Press, Chicago. 434 pp.

Brown, E. R., Q. Bone, K. P. Ryan, and N. J. Abbott. 1991. Morphology and electrical properties of Schwann cells around the giant axon of the squids *Loligo forbesi* and *Loligo vulgaris. Proceedings of the Royal Society of London Series B - Biological Sciences* 243:255-262.

Burgess, L. A. 1967. *Loliolus rhomboidalis*, a new species of loliginid squid from the Indian Ocean. *Bulletin of Marine Science* 17:319-329.

Camin, J. H. and R. R. Sokal. 1965. A method for deducing branching sequences in phylogeny. *Evolution* 19:311-326.

Carpenter, J. M. 1988. Choosing among multiple equally parsimonious cladograms. *Cladistics* 4:291-296.

Carpenter, J. M. 1994. Successive weighting, reliability and evidence. *Cladistics* 10:215-220.

Chrachri, A. and R. Williamson. 1993. Electrical coupling between primary hair cells in the statocyst of the squid, *Alloteuthis subulata. Neuroscience Letters* 161:227-231.

Cohen, A. C. 1976. The systematics and distribution of *Loligo* in the western North Atlantic, with description of two new species. *Malacologia* 15:229-367.

deMaintenon, M. J. 1990. *Comparative Morphology of the Cranium and Brachial Cartilage in the Cephalopod Suborder Myopsida (Cephalopoda: Teuthoidea).* Masters Thesis, University of Miami, Coral Gables, Florida. 63 pp.

de Queiroz, K. and J. Gauthier. 1990. Phylogeny as a central principle in taxonomy - phylogenetic definitions of taxon names. *Systematic Zoology* 39:307-322.

de Queiroz, K. and J. Gauthier. 1992. Phylogenetic taxonomy. *Annual Review of Ecology and Systematics* 23:449-480.

Drew, G. A. 1911. Sexual activities of the squid, *Loligo pealei* (Les.). I. Copulation, egg-laying and fertilization. *Journal of Morphology* 22:327-352.

Edwards, A. W. F. and L. L. Cavalli-Sforza. 1964. Reconstruction of evolutionary trees. *In: Phenetic and Phylogenetic Classification,* V. H. Heywood and J. MacNeill, eds. pp. 67-76. Systematics Association Publication 6, London.

Eernisse, D. J., J. S. Albert, and F. E. Anderson. 1992. Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan morphology. *Systematic Biology* 41:305-330.

Farris, J. S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* 18:374-385.

Farris, J. S. 1983. The logical basis of phylogenetic analysis. *In: Advances in Cladistics, Volume 2,* N. I. Platnick and V. A. Funk, eds. pp. 7-36. Proceedings of the Second Meeting of the Willi Hennig Society, Columbia University Press, New York.

Farris, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5:417-419.

Fields, W. G. 1965. The structure, development, food relations, reproduction, and life history of the squid *Loligo opalescens* Berry. *California Fish and Game Bulletin* 131:1-108.

Fishman, H. M. and J. Metuzals. 1993. Ca^{2+}-induced axosome formation in internally dialyzed giant axons of *Loligo pealei. Biological Bulletin* 185:292-293.

Fitch, W. M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. *Systematic Zoology* 20:406-416.

Gilbert, D. L., W. J. Adelman, Jr., and J. M. Arnold, eds. 1990. *Squid as Experimental Animals.* Plenum Press, New York. 516 pp.

Haneda, Y. 1963. Observations in the luminescence of the shallow-water squid *Uroteuthis bartschi. Science Report of the Yokosuka City Museum* 8:10-15.

Hanlon, R. T., S. v. Boletzky, T. Okutani, G. Perez-Gandaras, P. Sanchez, C. Sousa-Reis, and M. Vecchione. 1992. Suborder Myopsida. *In: "Larval" and Juvenile Cephalopods: a Manual for Their Identification,* M. J. Sweeney, C. F. E. Roper, K. M. Mangold, M. R. Clarke, and S. v. Boletzky, eds. pp. 37-53. Smithsonian Contributions to Zoology 513, Smithsonian Institution Press, Washington, D. C.

Hess, S. C. 1987. *Comparative Morphology, Variability, and Systematic Applications of Cephalopod Spermatophores (Teuthoidea and Vampyromorpha).* Doctoral Dissertation, University of Miami, Coral Gables, Florida. 581 pp.

Hillis, D. M. and J. P. Huelsenbeck. 1992. Signal, noise and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83:189-195.

Kluge, A. J. and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18:1-32.

Lee, P. G., P. E. Turk, W. T. Yang, and R. T. Hanlon. 1994. Biological characteristics and biomedical applications of the squid *Sepioteuthis lessoniana* cultured through multiple generations. *Biological Bulletin* 186:328-341.

Lu, C. C., C. F. E. Roper, and R. W. Tait. 1985. A revision of *Loliolus* (Cephalopoda: Loliginidae), including *L. noctiluca,* a new species of squid from Australian waters. *Proceedings of the Royal Society of Victoria* 97:59-85.

Lu, C. C. and R. W. Tait. 1983. Taxonomic studies on *Sepioteuthis* Blainville (Cephalopoda: Loliginidae) from the Australian region. *Proceedings of the Royal Society of Victoria* 95:181-204.

Maddison, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* 40:315-328.

Maddison, W. P. 1993. Missing data versus missing characters in phylogenetic analysis. *Systematic Biology* 42:576-581.

Maddison, W. P., M. J. Donoghue, and D. R. Maddison. 1984. Outgroup analysis and parsimony. *Systematic Zoology* 33:83-103.

Maddison, W. P. and D. R. Maddison. 1992. *MacClade: Analysis of Phylogeny and Character Evolution. Version 3.04.* Sinauer Associates, Sunderland, Massachusetts.

McFall, M. and E. G. Ruby. 1991. Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial symbiosis. *Science* 254:1491-1494.

McGowan, J. A. 1954. Observations on the sexual behavior and spawning of the squid, *Loligo opalescens*, at La Jolla, California. *California Fish and Game* 40:47-54.

Morejohn, G. V., J. T. Harvey, and L. T. Krasnow. 1978. The importance of *Loligo opalescens* in the food web of marine vertebrates in Monterey Bay, California. *In: Biological, Oceanographic, and Acoustic Aspects of the Market Squid,* Loligo opalescens *(Berry),* C. W. Recksiek and H. W. Frey, eds. pp. 67-98. California Department of Fish and Game Fish Bulletin 169.

Nateewathana, A. 1992. Taxonomic studies on loliginid squids (Cephalopoda: Loliginidae) from the Andaman Sea coast of Thailand. *Phuket Marine Biological Center Research Bulletin* 57:1-40.

Natsukari, Y. 1976. Taxonomic and morphological studies on the loliginid squids - II. Description and new record of *Loligo sibogae* (Adam, 1954) from Formosa. *Venus* 35:15-23.

Natsukari, Y. 1983. Taxonomical and morphological studies on the loliginid squids - III. *Nipponololigo,* a new subgenus of the genus *Loligo. Venus* 42:313-318.

Natsukari, Y. 1984. Taxonomical and morphological studies on the loliginid squids - IV. Two new genera of the family Loliginidae. *Venus* 43:229-239.

Natsukari, Y. and T. Okutani. 1975. Taxonomical and morphological studies on the loliginid squids - I. Identity of *Loligo chinensis* Gray, 1849, redescription of the type specimen and taxonomic review (Cephalopoda: Loliginidae). *Venus* 34:85-91.

Nesis, K. N. 1987. *Cephalopods of the World.* T. F. H. Publications, Neptune City, New Jersey. 351 pp.

Patterson, C. 1982. Morphological characters and homology. *In: Problems of Phylogenetic Reconstruction,* K. A. Joysey and A. E. Friday, eds. pp. 21-74. Academic Press, New York.

Preuss, T. and B. U. Budelmann. 1995. Proprioceptive hair cells in the neck of the squid *Lolliguncula brevis* - a sense organ in cephalopods for the control of head-to-body position. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences* 349:153-178.

Pringennies, D. and J. M. Jørgensen. 1994. Morphology of the luminous organ of the squid *Loligo duvauceli* d'Orbigny, 1839. *Acta Zoologica* 75:305-309.

Rehder, H. A. 1945. A new genus and species of squid from the Philippines. *Proceedings of the Biological Society of Washington* 58:21-26.

Roper, C. F. E. 1969. Systematics and zoogeography of the worldwide bathypelagic squid *Bathyteuthis. Bulletin of the United States National Museum* 291:1-210.

Roper, C. F. E., M. J. Sweeney, and C. E. Nauen. 1984. *Cephalopods of the World. An Annotated and Illustrated Catalogue of Species of Interest to Fisheries.* FAO Fisheries Synopsis 125, Volume 3, 277 pp.

Rosenthal, J. J. C. and W. F. Gilly. 1993. Amino acid sequence of a putative sodium channel expressed in the giant axon of the squid *Loligo opalescens. Proceedings of the National Academy of Sciences of the United States of America* 90:10026-10030.

Sober, E. 1988. *Reconstructing the Past: Parsimony, Evolution and Inference.* MIT Press, Cambridge, Massachusetts. 265 pp.

Suter, S. J. 1994. Cladistic analysis of the living cassiduloids (Echinoidea), and the effects of character ordering and successive approximations weighting. *Zoological Journal of the Linnean Society* 112:363-387.

Swofford, D. L. 1993. *PAUP: Phylogenetic Analysis Using Parsimony. Version 3.1.1.* Illinois Natural History Survey, Champaign.

Swofford, D. L., and G. L. Olsen. 1990. Phylogeny reconstruction. *In: Molecular Systematics,* D. M. Hillis and C. Moritz, eds. pp. 411-501. Sinauer Associates, Sunderland, Massachusetts.

Toll, R. B. 1982. *The Comparative Morphology of the Gladius in the Order Teuthoidea (Mollusca: Cephalopoda) in Relation to Systematics and Phylogeny.* Doctoral Dissertation, University of Miami, Coral Gables, Florida. 390 pp.

Vecchione, M., T. F. Brakoniecki, Y. Natsukari, and R. T. Hanlon. In press. A provisional generic classification of the family Loliginidae. *Smithsonian Contributions to Zoology.*

Voss, G. L. 1953. A new family, genus and species of myopsid squid from the Florida Keys. *Bulletin of Marine Science of the Gulf and Caribbean* 2:602-609.

Voss, G. L. 1963. Cephalopods of the Philippine Islands. *United States National Museum Bulletin* 234, 180 pp.

Voss, G. L. 1974. *Loligo surinamensis*, a new species of loliginid squid (Cephalopoda, Myopsida) from northeastern South America. *Zoologische Mededelingen* 48:43-53.

Voss, G. L. 1977. Present status and new trends in cephalopod systematics. *In: The Biology of Cephalopods,* M. Nixon and J. B. Messenger, eds. pp. 49-60. Academic Press, New York.

Watrous, L. E. and Q. Wheeler. 1981. The outgroup comparison method of character analysis. *Systematic Zoology* 30:1-11.

Wiens, J. J. 1995. Polymorphic characters in phylogenetic systematics. *Systematic Biology* 44:482-500.

Wiley, E. O., D. Siegel-Causey, D. R. Brooks, and V. A. Funk. 1991. *The Compleat Cladist - a Primer of Phylogenetic Procedures.* University of Kansas Museum of Natural History Special Publication 19, Lawrence, Kansas. 158 pp.

Wilkinson, M. 1995. Coping with abundant missing entries in phylogenetic inference using parsimony. *Systematic Biology* 44:501-514.

Williams, L. W. 1909. *The Anatomy of the Common Squid* Loligo pealii, *Lesueur.* E. J. Brill Library and Printing Office, Leiden, Holland. 92 pp.

Yeatman, J. and J. A. H. Benzie. 1994. Genetic structure and distribution of *Photololigo* spp. in Australia. *Marine Biology* 118:79-87.

Young, J. Z. 1938. The functioning of the giant nerve fibres of the squid. *Journal of Experimental Biology* 15:170-185.

Young, J. Z. 1991. *Ctenopteryx* the comb-fin squid is related to *Loligo. Bulletin of Marine Science* 49:148-161.

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APPENDIX I - Data Matrix. Inapplicable characters for particular taxa are indicated by "n", while missing or unknown character states are coded as "?". Certain taxa are polymorphic for particular characters. In these cases, the following code was used: "a" = states 0 and 1, "b" = states 1 and 2, "c" = states 0 and 2, and "d" = states 1 and 3. Taxon names above the species level are based on Nesis (1987).

	111111111122222222223333333333444444444 123456789012345678901234567890123456789012345678
Todarodes pacificus	11?10102011010100n103nnnnnnnn2000????????2000010
Rossia	00?1a??0?110?1?20n001nnnnnnnn0111????????0011101
Moroteuthis robusta	11?00nn3?2???0??0n000nnnnnnnn2000?????2002?00010
Sepia	00?01nn200??01?01?002nnnnnnnn1nn10???????n011011
Ctenopteryx	???11??0nnnnn0??1?010nnnnnnnn3nn1?????20?0?00010
Bathyteuthis	00?11110nnnnn0?011100nnnnnnnn0110?????20?0?00010
Euprymna	00?0ann0nnnnn1?10?021nnnnnnnn01100???????0011101
Pickfordiateuthis pulchella	110a0?31?0???0?20?0021266?00?01110001?1000?11010
Loligo opalescens	11?101120110a000110020200?00?20010000a???0011010
Loligo pealei	11110112011110?0110021200?01?2001?000a0110011010
Loligo ocula	11?10112?11110?01?0021210?01?2001?0000???0?11010
Loligo plei	11110112011110?0110020110?01?2001100000110011010
Loligo roperi	11?10112011010?01?0020210?01?2001000000110011010
Loligo surinamensis	11?10112011110?01?0021210?00?2001?00000110?11010
Loligo sanpaulensis	11?101220110100010002001??01?2001?0000?100?11010
Loligo gahi	11?101120110100010002001??01?2001100000110011010
Loligo vulgaris	11?101120100?0?0110020111?00?200110000????011010
Loligo reynaudii	11?10a12?a1010?01?00201bb?00?2001?0000011??11010
Loligo forbesi	11?10132?110?0?0110020111?01?2001?0000?????11010
Loligo bleekeri	11?10??2?????0?01?0020100?00?200110000?????11010
Loligo duvauceli	11?10112111010001?0320111100?2001?2200?1?0011010
Loligo edulis	11?1011211111000100320122100?200112200?1??011010
Loligo chinensis	11?10102111110?0110320122000?200101100?1??011010
Loligo vossi	11?10012011010?01?002011110??20011?????????11010
Loligo japonica	11?10112011010001?0020154?0012001?1100????011010
Loligo beka	11?101b2011010?01?0020154?0012001?1100?1???11010
Loligo sumatrensis	11?10112010010?01?0020154?0??20010000??1??011010
Loligo uyii	11?1012200??10001?0020154?0012001?1100?????11010
Loligo sibogae	11?101121110?0001?0320122100?20011???0?1??011010
Loligo singhalensis	11?101a2111110?01?0320111100?2001?00001100011010
Loligo pickfordae	11?10012?110?0?01?03201??????20011????????011010
Loligo reesi	11?10112111010?01?0320111?10?20011000?0110011010
Loligo kobiensis	11?1012200??10001?0020154?0012001?02100110011010
Alloteuthis media	11?1011201aa10100n00201bb100?212100010???1011010
Alloteuthis subulata	11?1011201aa10100n00201bb100?2121000100111011010
Alloteuthis africana	111101120110?0100n00201bb100?212100010???1011010
Loliolus hardwickei	1101012211001000110022354?0002101022?02110111010
Loliolus affinis	11?101221100?0001?0022354?c0021010???0?110111010
Loliolus noctiluca	11?1011200??0000100322311?10?21010???01110011010
Sepioteuthis sepioidea	11010112110010000n0020111200?1nn100020???0011010
Sepioteuthis lessoniana	11?1000211101000110020111200?1nn1000001110011010
Sepioteuthis australis	11?10002111010001100201112?0?1nn100000011?011010
Lolliguncula argus	11?101120110?0?00n00300d??a0?2111010030100011010
Lolliguncula mercatoris	11?10022?110?0?00n0020133301?211102202?????11010
Lolliguncula brevis	11?10122111010001?0020a3a?a0?2111000010100011010
Lolliguncula panamensis	11?10122111aa00011002003??10?2111000020100011010
Uroteuthis bartschi	112101121110?0001?0320101000?2021100000111011010
Loliolopsis diomedeae	111101120110?0?01?0020135?10?211100001???0011010

APPENDIX II. Character Descriptions and Codings. Supplemental sources of information used are listed by each character (or each character suite) with the exception of particular references that pertain to certain taxa, including: *Bathyteuthis* (Roper, 1969); *Pickfordiateuthis pulchella* (see Voss, 1953; Brakoniecki, 1996); *Alloteuthis africana* (see Adam, 1950) *Loligo pealei, L. ocula, L. plei,* and *L. roperi* (see Cohen, 1976); *L. sanpaulensis* and *L. gahi* (see Brakoniecki, 1984); *L. chinensis* (see Natsukari and Okutani, 1975; Nateewathana, 1992); *L. edulis, L. beka, Loliolus affinis, Loligo sumatrensis* (see Nateewathana, 1992); *L. surinamensis* (see Voss, 1974); *L. kobiensis/Loliolus rhomboidalis* (see Burgess, 1967); *Loligo sibogae* (see Adam, 1954; Natsukari, 1976); *L. pickfordae, L. duvauceli, L. singhalensis* (see Adam, 1954); *Loliolus* (Lu *et al.*, 1985); *Sepioteuthis* (Lu and Tait, 1983); *Lolliguncula argus* (see Brakoniecki and Roper, 1985); *L. panamensis* (see Berry, 1911; Brakoniecki, 1980); *Uroteuthis bartschi* (see Adam, 1954; Rehder, 1945; Voss, 1963), and *Loliolopsis diomedeae* (see Berry, 1929). Characters are grouped by system; numbers refer to the position of the character in the data matrix (Appendix I).

A. Radula

1. Rachidian tooth (unicuspid/tricuspid) - Within most squids, the radula is comprised of seven longitudinal rows of teeth – a central rachidian, a pair of first and second lateral teeth, and a pair of marginal teeth. The rachidian tooth is usually either composed of a single cusp (unicuspid), or has a large central tooth with two lateral cusps (tricuspid).
2. Lateral teeth (unicuspid/bicuspid) - The first and second lateral teeth within loliginids are comprised of two cusps, while the first and second lateral teeth in numerous other squids are unicuspid.

B. Arm/tentacle club/sucker rings

3. Brachial cartilage (absent/fibrous type/hyaline type) (deMaintenon, 1990) - The brachial cartilage is a small cartilaginous structure found in many squids located antero-ventrally to the cranial cartilaginous body. The brachial cartilage seems to serve as a base for the tentacles and fourth arms. Some loliginids (*e.g. Sepioteuthis sepioidea*) lack this structure altogether, some have either a variable region of fibrous connective tissue (coded as "fibrous type"), and still others possess a distinct block of hyaline cartilage ("hyaline type").
4. Arm sucker rings (smooth/with teeth) - All taxa in this analysis possess horny chitinous rings in their arm suckers. These rings are either smooth, or they possess teeth of various shapes.
5. Arm sucker rows (two/four) - All loliginids and many other squids possess two rows of stalked suckers running along the arms. Many cephalopods possess four rows of suckers along the inner surface of the arms.
6. Arm sucker teeth position (all around ring/only on distal edge) - In most loliginids, the teeth on the chitinous sucker rings of the large proximal suckers on the third and fourth arms are found only on the distal edge of the sucker rings. In particular species, the sucker ring teeth are found all around the ring (although decreasing in size in the proximal region of the ring).
7. Arm sucker teeth shape (sharp/square or rounded and blunt/low, wide and flat/small, low and rounded) - A great diversity of arm sucker tooth shape can be found among loliginid species. Teeth are generally either tall, slender, and sharply pointed (as in *Loligo chinensis*), tall with rounded tips, or relatively flat and wide (often considerably wider than tall). Some (such as *L. japonica*) possess low rounded teeth, usually slightly wider than tall, with rounded, half-circle edges. The lone specimen of *L. forbesi* examined possessed a unique tooth morphology, consisting of very small, irregular teeth, giving the ring a pebbly appearance.
8. Club morphology (many tiny suckers/two rows in manus/four rows in manus/no marginal suckers or distinct dactylus) - The number of sucker rows in the manus region of the tentacle clubs is variable among squids. Many taxa (such as *Bathyteuthis*) possess a large number of minuscule suckers on the tentacle clubs, with no distinct regions. Other squids possess a distinct carpus, manus and dactylus, with two rows of suckers in the manus region. All loliginid squids possess a distinct manus and dactylus, with four rows of suckers (two central rows and two outer marginal rows) in the manus. *Pickfordiateuthis* possesses a few large, central suckers in the manus, with no marginal suckers and no distinct dactylus.
9. Central club sucker size (much larger than marginal suckers/similar in size to marginal suckers) - There is substantial variation in the size of the central club suckers relative to that of the marginal club suckers. In some loliginid species, marginal club suckers are nearly as large as central suckers while, in others, the marginal suckers are considerably smaller than the nearby central suckers.
10. Central manus sucker teeth (absent/present/hooks) - Some loliginid species possess smooth, toothless chitinous rings in their largest central club suckers. Most loliginids have teeth of some kind on their central manus sucker rings. Some outgroup taxa possess sharp hooks in their club suckers.
11. Central manus sucker teeth shape (blunt/pointed) - Central club sucker teeth are generally sharp and pointed, but some species have central manus suckers with teeth with rounded or blunt tips.
12. Central manus sucker teeth pattern (uniform sizes/many with alternating small and large teeth) - Patterns in central club sucker teeth sizes are variable, even among suckers on one tentacle club. In general, however, teeth are subequal in size on each individual ring. In some species, teeth show an alternating pattern (often large-small-large-small). Some species show more complex patterns of alternating small, medium and large teeth.
13. Marginal manus sucker teeth shape (blunt/pointed).
14. Retractile tentacles (absent/present).
15. Trabeculae number per marginal club sucker (one per marginal sucker/two per marginal sucker) - Most loliginid species possess thick trabeculae (muscular supports for the protective membranes of the tentacle clubs) spaced evenly between the marginal club suckers, averaging one trabecula per marginal sucker. Other species (members of the genus *Alloteuthis*) possess two trabeculae (Roper *et al.*, 1984) attached near the base of each marginal sucker.

C. Buccal lappets

16. Buccal lappet lobes (seven/eight/no lobes) - The number of buccal lappet lobes is variable among squids, and has been used as a taxonomic character. All loliginid squids possess seven buccal lappet lobes.
17. Buccal lappet suckers (absent/present) - Most loliginid species have tiny suckers on the inner surface of their buccal lappets. The three species of *Alloteuthis* and *Sepioteuthis sepioidea* do not have suckers on their buccal lappets.
18. Buccal lappet sucker teeth (absent/present).
19. Buccal membrane formula (DDVV/DDVD) - The location of the buccal lappet supports relative to the arms has commonly been used in cephalopod systematic studies. In loliginids and many other squid groups, the buccal lappet supports are attached to the

dorsal edges of the first and second arms, and to the ventral edges of the third and fourth arms (this pattern is often abbreviated "DDVV"). In other squids, the supports are attached to the dorsal edge of the first, second and fourth arms, and to the ventral edges of the third arms (abbreviated "DDVD").

D. Photophore morphology

20. Photophores on ink sac (absent/one round photophore/one U-shaped photophore/two bean-shaped photophores) - Photophores (bioluminescent organs) of various types are widespread throughout many cephalopod taxa. Most taxa examined in this study lack photophores. Some (*Ctenopteryx*) possess a single large round photophore on the ink sac. Others (*Euprymna*) possess a large U-shaped photophore on the ventral surface of the ink sac. Some loliginid species possess two oval or bean-shaped photophores. These species have been grouped in three separate genera (*Loligo, Uroteuthis,* and *Loliolus*) by earlier authors, while recent workers have suggested that loliginids with photophores constitute a natural group (named *Photololigo*). Because photophore shape and number varies across Loliginidae, *Ctenopteryx,* and *Euprymna,* these structures have been coded as different states. Because photophore number and basic external morphology is similar across all loliginid species with photophores, these structures have been coded as putative homologues for this analysis. Only through cladistic analysis of many characters can individual statements of homology such as this be assessed (the test of congruence; Patterson, 1982).

E. Hectocotylus morphology (Brakoniecki, 1986)

21. Hectocotylized arms (none/left dorsal arm/left ventral arm/right ventral arm) - The hectocotylus is a modified arm (or arms) in males that aids in transfer of spermatophores to the female. Hectocotyluses can exhibit radically different sucker morphology from the other arms, or can be of a very different length from the rest of the arms. Different cephalopod taxa have different arms hectocotylized, or lack an obvious hectocotylus altogether.
22. Modified region in dorsal row of hectocotylus (distal suckers modified to tip of arm/central suckers only modified/all suckers modified) - The region of modified suckers in the dorsal row of the hectocotylus is variable across loliginid species. In most species, only the distalmost suckers show any sort of modification, extending to the tip of the arm. A few species (*Loliolus*) show sucker modification along the entire length of the arm. Other species show minimal sucker differentiation on the hectocotylus which is restricted to a central region of the arm.
23. Modified region in ventral row of hectocotylus (distal suckers modified to tip of arm/central suckers only modified/all suckers modified) - See description for character 22.
24. Type of sucker morphology in dorsal row (small suckers with large pedicels/tiny suckers with long triangular pedicel/robust conical suckerless papillae/long thin suckerless papillae/tiny papillae/small suckers and stalks) - Sucker modifications are extremely variable in loliginid hectocotyluses, but seem to fit into a few distinct classes, which may be related to one another in complex ways. Some hectocotyluses possess small suckers at the tip of large, thick, columnar stalks (pedicels). Others show a similar modification - tiny suckers at the tip of pedicels which are distinctly wider at the base than at the tip, giving them a triangular shape. Some species possess thick, conical "papillae" that appear to lack suckers of any kind, but come to a point at their tips. Others possess a similar, but distinct, sucker modification in which long, rounded finger-like papillae are found. Some species possess only minute papillae in the dorsal row of the hectocotylus. Finally, a few species have suckers that are slightly smaller than normal, but are otherwise unmodified.
25. Type of sucker morphology in ventral row (small suckers with large pedicel/tiny suckers with long triangular pedicel/robust conical suckerless papillae/long thin suckerless papillae/fused crest/no suckers/suckers embedded in swelling) - Most sucker modifications found in the dorsal row of the hectocotylus are also found in the ventral row. There are a few differences. In many species, the ventral row of suckers is present as a row of tiny papillae, similar in morphology to the "finger-like" papillae described above, but much smaller. In some species, the pedicels of the ventral sucker row are fused with the ventral protective membrane, resulting in a series of thickened slabs (a fused crest) in the ventral row. One species (*Loliolopsis diomedeae*) completely lacks suckers of any kind in the ventral row. The ventral row of suckers in *Pickfordiateuthis* appears to be embedded in a swelling, an autapomorphy of this taxon.
26. Size of suckers on hectocotylus (suckers of both rows about the same size/ventral row suckers larger/dorsal row suckers larger/dorsal row suckers larger proximally, ventral row suckers larger distally) - In many cases where the sucker modifications in both rows are the same, consistent differences in sucker height can be seen between the rows. In some cases, the suckers in each row are approximately equal in size, tapering to the tip of the arm. Alternatively, the suckers in either the dorsal or ventral row can be larger than adjacent suckers in the other row. In a few species, dorsal row suckers appear to be larger proximally, but rapidly decrease in size down the length of the arm, while suckers in the ventral row either increase in size, or decrease much more slowly, resulting in the dorsal row of suckers being larger proximally, but the ventral rows of suckers being larger distally.
27. Length of hectocotylus (same length as fellow arm/longer than fellow arm/shorter than fellow arm) - In most cases, the length of the hectocotylized ventral arm is approximately the same as the length of the non-hectocotylized ventral arm. In some cases, however, the hectocotylized arm is distinctly longer or shorter than the other ventral arm.
28. Ridge between sucker rows in modified region of hectocotylus (absent/present) - A fleshy ridge is evident between the sucker rows in the modified portion of the hectocotylus in some loliginid species. This ridge is lacking in males of most loliginid species.
29. Fused crest in ventral row (without suckers/with suckers) - In squids with a fused crest ventral row modification, some species have suckers at the tops of the crest, while others (*Loliolus hardwickei, L. affinis*) have a suckerless fused crest.

F. Fin morphology

30. General fin shape (subterminal and round/terminal, longitudinally oval/terminal, rhomboid, or transversely oval/longitudinally oval and trabeculate) - The general shape of the swimming fins on the mantle of cephalopods is highly variable. Fin morphology for the species in this study can be split into four groups. Some outgroup species possess small, round, or kidney-shaped subterminal fins. Some species (*Sepia, Sepioteuthis*) possess fins which extend from almost the anterior edge of the mantle to the posterior tip, and are shaped like half-ovals. *Ctenopteryx* possesses longitudinally oval, trabeculate fins that are rather distinct from the fins of other squids. Most loliginid species have terminal fins whose anterior attachment point is far from the anterior edge of the mantle. These fins are either rhomboid or transversely oval in shape.
31. Anterior fin edge (nearly straight/convex).

32. Posterior fin edge (straight/convex/concave, longer than anterior edge).

G. Sexual morphology

33. Accessory nidamental glands (absent/present).
34. Cutaneous ridge on ventral surface of mantle in males (absent/present) - Mature males of some loliginid species possess a robust, serrated ridge running the length of the ventral midline of the mantle. Males of most loliginid species lack this feature.
35. Male arm II sucker size (normal/proximal suckers enlarged/all suckers enlarged) - Males of particular loliginid species have larger suckers (either proximally, or along the entire length of the arm) on their second (dorsolateral) arm pair than females of similar size of the same species.
36. Male arm III sucker size (normal/proximal suckers enlarged/all suckers enlarged) - See description for character 35.
37. Male right arm IV sucker size (normal/proximal suckers enlarged/proximal suckers reduced) - As described in character 35, males of some loliginid species show enlargement (or reduction) of the proximal suckers of the right arm IV suckers relative to the proximal suckers on the hectocotylus (left arm IV).

H. Spermatophores (Hess, 1987)

38. Spermatophore placement (onto buccal membrane/near left gill on mantle wall/on buccal membrane and left gill/on buccal membrane and right gill) - Clusters of deposited spermatophores can often be found during dissections of females. The location of these clusters varies across species. Females in most species possess a spermatophore receptacle on the buccal membrane near the mouth. In some well-studied species, however, spermatophores have been found attached to the buccal receptacle and to the base of either the left or right gill. In particular species, toughened "spermatophoric pads" can be found on the inside of the mantle cavity near the left gill where spermatophores are attached. Lu *et al.* (1985) reported that some females of *Loliolus noctiluca* also possess spermatophoric pads, and other authors have seen spermatophores placed on the left side of the inner mantle wall in *Loligo opalescens* and *L. pealei* (see Drew, 1911; McGowan, 1954; Fields, 1965). This character needs to be reviewed further, and may prove to be variable across several (or most) loliginid species, potentially limiting its usefulness in cladistic analysis.
39. Spermatophore cement body ratio (oral portion longer than aboral portion/oral and aboral portions approximately equal in length/oral portion smaller than aboral portion) - Many of these data (and data for characters 40 and 41) have been coded directly into the matrix from Hess (1987).
40. Spiral filament in spermatophore (absent/present).
41. Oral component of spermatophore cement body (not divided/divided).

I. Miscellaneous

42. "Conus" (absent/present with edges fused/present, with edges unfused) - In species which possess internal, non-calcified shell remnants (pens or gladii), some possess a "secondary conus" (Toll, 1982) in which the posterior edges of the gladius are fused around the posterior visceral mass to form a cone. In some loliginids, the posterior edges of the gladius are curled ventrally and actually overlap ventrally, but are not fused. Most loliginids possess gladii which show only moderate ventral curling posteriorly (they lack a "conus").
43. Papillae on ink sac of males (absent/present) - Research on the genus *Loliolus* (Lu *et al.*, 1985) has shown that males of two species possess small pores on the ink sac. This characteristic has not been reported in any other loliginid species, and was not found in males of any other species examined in this study.
44. Cornea (absent/present) - The presence or absence of a corneal covering over the eye has been the nominal character separating the oegopsid squids from the myopsid squids.
45. Oviducts (both developed/only left oviduct developed).
46. Muscular septum in mantle cavity (absent/present) - Certain outgroup taxa (*Rossia, Euprymna*) possess a muscular septum dividing the mantle cavity longitudinally into two halves. Loliginids and other taxa in this study lack this feature.
47. Nuchal cartilage (absent/present).
48. Digestive gland (single/paired).

APPENDIX III. Material Examined. Material examined is listed by species name, ingroups first, in alphabetical order. The sex and approximate dorsal mantle length, when known, are listed for each specimen examined. (CAS, California Academy of Sciences; DML, dorsal mantle length; F, female; J, juvenile (sex not determined); M, male; NMNH, United States National Museum of Natural History; U, sex undetermined; UMML, University of Miami Invertebrate Museum).

Ingroup taxa

Alloteuthis africana Adam, 1950 - NMNH 727426 (1 M, 56 mm DML), NMNH BCF Table 6IX 6E-2-218 9-6-63 (2 M, 78 and 71 mm DML), UMML 1757 (1 F, 45 mm DML; 1 M, 58 mm DML).

A. media (Linné, 1758) - NMNH 817475 (3 F, 56, 64, and 67 mm DML; 2 M, 42 and 50 mm DML), UMML 1251.

A. subulata (Lamarck, 1798) - UMML 1252 (2 M, 100 and 101 mm DML), NMNH 817534 (1 F, 70 mm DML).

Loligo beka Sasaki, 1929 - UMML 1209 (1 F, 55 mm DML), UMML 1210 (1 M, 59 mm DML).

L. bleekeri Keferstein, 1866 - NMNH 332905 (1 J, 40 mm DML), UMML 1211 (2 M, 36 and 38 mm DML).

L. budo (Wakiya and Ishikawa, 1921) - UMML 1212 (1 F, 170 mm DML; 1 M, 190 mm DML).

L. chinensis Gray, 1849 - UMML PJ-102 (2 F, 75 and 107 mm DML), UMML PJ-110 (1 F, 92 mm DML).

L. duvauceli Orbigny, 1848 - NMNH 817827 (2 F, 100 and 123 mm DML), NMNH 817829 (1 M, 126 mm DML), NMNH 727560 (1 F, 110 mm DML), NMNH 727561 (2 M, 70 and 93 mm DML), NMNH 817823 (1 M, 66 mm DML), CAS 084583.

L. edulis Hoyle, 1885 - NMNH 814158 (4 M, 127, 133, 136, and 142 mm DML), CAS 030539 (2 M, 99 and 107 mm DML).

L. etheridgei (Berry, 1918) - UMML 1220 (1 F, 90 mm DML; 1 M, 104 mm DML).

L. forbesi Steenstrup, 1856 - NMNH (1 F, 133 mm DML).

L. gahi Orbigny, 1835 - UMML 2087 (1 F, 72 mm DML), UMML

2090 (2 F, 90 and 91 mm DML; 1 M, 69 mm DML).

L. japonica Hoyle, 1885 - NMNH 727551 (2 M, 75 and 77 mm DML), NMNH 332903 (3 M, 58, 70, and 77 mm DML), UMML 1224 (2 M, 61 and 68 mm DML), UMML 1226 (1 F, 60 mm DML).

L. kobiensis Hoyle, 1885 - UMML 31.2203 (1 F, 87 mm DML; 1 M, 76 mm DML).

L. ocula Cohen, 1976 - UMML 1683 (2 M, 53 and 62 mm DML), NMNH 727095 (2 M, 87 and 127 mm DML) (paratypes), NMNH 727096 (1 F, 89 mm DML).

L. patagonica (Smith, 1881) - UMML 1231 (1 F, 83 mm DML).

L. pealei LeSueur, 1821 - NMNH 730069 (2 M, 85 and 95 mm DML), NMNH 730531, NMNH 730183 (1 M, 206 mm DML), NMNH 814169 (1 F, 136 mm DML), NMNH 814191 (1 M, 90 mm DML; 1 J, 83 mm DML).

L. plei Blainville, 1823 - NMNH 574548 (1 M, 105 mm DML), NMNH 576456 (4 M, 146, 154, 195, and 217 mm DML), NMNH 813979 (2 M, 181 and 260 mm DML), NMNH 814288 (1 F, 120 mm DML; 1 M, 105 mm DML), NMNH 814316 (1 M, 198 mm DML), NMNH 814317 (1 M, 213 mm DML), NMNH 814318 (1 M, 197 mm DML), NMNH 814315 (1 M, 163 mm DML), NMNH 574320 (1 M, 169 mm DML), NMNH 574180 (2 M, 215 and 277 mm DML).

L. reesi (Voss, 1963) - UMML 1803 (1 M, 62 mm DML).

L. reynaudi Orbigny, 1845 - UMML 1233 (1 M, 175 mm DML), UMML 1234 (1 M, 95 mm DML).

L. roperi Cohen, 1976 - NMNH 575874 (1 M, 53 mm DML), UMML 933 (1 F, 38 mm DML; 2 M, 41 and 43 mm DML) (paratypes), UMML 1798 (1 M, 55 mm DML), UMML 72777 (1 M, 77 mm DML) (holotype).

L. sanpaulensis Brakoniecki, 1984 - UMML 1813 (2 M, 144 and 150 mm DML) (paratypes).

L. sibogae (Adam, 1954) - NMNH 575813 (1 F, 123 mm DML; 1 M, 139 mm DML).

L. singhalensis Ortmann, 1891 - UMML 31.2323 (1 M, 140 mm DML), UMML 2168 (1 M).

L. sumatrensis Orbigny, 1835 - NMNH 817821 (1 F, 52 mm DML), NMNH 817820 (1 F, 53 mm DML; 2 M, 48 and 50 mm DML).

L. surinamensis Voss, 1974 - UMML 2053 (1 F, 92 mm DML), UMML 31.2023 (2 F, 76 and 88 mm DML).

L. uyii Wakiya and Ishikawa, 1921 - CAS 035049, UMML 1239 (1 F, 94 mm DML; 1 M, 69 mm DML).

L. vossi (Nesis, 1982) - UMML 1259 (2 M, 65 and 78 mm DML).

L. vulgaris Lamarck, 1798 - UMML 1240 (1 M, 210 mm DML), UMML 1241 (1 F, 43 mm DML), UMML 1597 (1, 137 mm DML).

Loliolopsis diomedeae (Hoyle, 1904) - CAS 030492 (2 M, 38 and 41 mm DML), NMNH 576907 (2 F, 90 and 93 mm DML), NMNH 730085, UMML 31.697 (1 F, 102 mm DML), UMML (2 F, 95 and 104 mm DML), UMML 1799 (1 M, 83 mm DML).

Loliolus affinis (Steenstrup, 1856) - CAS 030250 (2 M, 21 and 25 mm DML).

L. hardwickei (Gray, 1849) - CAS 030251 (1 M, 40 mm DML), NMNH 817822.

L. noctiluca Lu, Roper, and Tait, 1985 - NMNH 00813974 (1 F, 68 mm DML; 3 M, 50, 51, and 56 mm DML).

Lolliguncula argus (Brakoniecki and Roper, 1985) - CAS 030252 (2 F, 43 and 43 mm DML; 1 M, 39 mm DML).

L. brevis (Blainville, 1823) - CAS 030491 (2F, 42 and 43 mm DML), NMNH 884122 (1 M, 66 mm DML).

L. mercatoris Adam, 1941- UMML 1244 (1 M), UMML 31.790 (1 M, 15 mm DML), UMML 31.2550 (1 M, 21 mm DML).

L. panamensis Berry, 1911 - CAS 030157 (1 M, 44 mm DML), CAS 030495 (2 F, 86 and 105 mm DML).

Pickfordiateuthis pulchella (Voss, 1953) - UMML 1948 (20 mm DML).

Sepioteuthis australis Quoy and Gaimard, 1832 - NMNH 816311 (1 F, 102 mm DML).

S. lessoniana Lesson, 1830 - CAS 030624 (2 F, 93 and 105 mm DML), NMNH 297637 (2 M, 127 and 166 mm DML), NMNH CH6-7 (1 M, 155 mm DML).

S. loliginiformes (Rüppell and Leuckart, 1828) - NMNH 730575 (1, 17 mm DML).

S. sepioidea (Blainville, 1823) - CAS 030428 (1 M, 72 mm DML), NMNH 576881 (1 M, 101 mm DML), NMNH 9548 (2 M, 99 and 106 mm DML), NMNH 576877 (1 M, 110 mm DML), NMNH 814382 (1 F, 119 mm DML).

Uroteuthis bartschi Rehder, 1945 - CAS 030485 (1 M, 104 mm DML), NMNH 575388 (1 M, 122 mm DML), UMML 1255 (2 F, 119 and 121 mm DML).

Outgroup taxa

Bathyteuthis berryi Roper, 1968 - NMNH 727573 (1 M, 47 mm DML).

Ctenopteryx sicula (Vérany, 1851) - NMNH 728929, NMNH 727721, NMNH 730695 (1 U, 68 mm DML), NMNH 728935 (2 U, 21 and 45 mm DML), NMNH 730696 (1 U, 75 mm DML), NMNH 730697 (1 M, 81 mm DML), NMNH 730698 (1 F, 52 mm DML).

Euprymna moresi (Verrill, 1881) - CAS 021433 (1 F, 31 mm DML; 1M, 33 mm DML).

E. scolopes (Berry, 1913) - CAS 030512 (2 U, 24 and 28 mm DML), CAS 030751 (1 U, 30 mm DML).

Rossia pacifica Berry, 1911 - CAS 030356 (2 F, 30 and 30 mm DML), CAS 081003 (1 U, 50 mm DML).

Sepia aculeata Orbigny, 1848 - CAS 084742 (1 M, 210 mm DML).

Moroteuthis robusta (Dall in Verrill, 1876) - CAS 030111 (partial specimen, total length 9 ft., 7 inches), CAS 035031 (1 U, 300+ mm DML).

Todarodes pacificus (Steenstrup, 1880) - CAS 024414 (2 U, 151 and 155 mm DML), CAS 024415 (1 U, 166 mm DML), CAS 030961, CAS 031020 (1 U, 106 mm DML).

Biochemical study of the population heterogeneity and distribution of the oval squid *Sepioteuthis lessoniana* complex in southwestern Japan

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Abstract: The three taxa of the *Sepioteuthis lessoniana* (Lesson, 1830) complex, AKAIKA, SHIROIKA, and KUAIKA, are genetically and reproductively independent and coexistent without hybridizing along the coast of Ishigaki Island, Okinawa. The present study analyzed 554 specimens of *Sepioteuthis* collected from 11 localities in inshore waters of southwestern Japan to elucidate the distributional patterns and population structures by means of horizontal starch gel electrophoresis at 13 genetic loci encoding for ten enzymes. The results showed that each of the three taxa has a different distributional pattern. SHIROIKA is widely distributed in the tropical to warm temperate regions throughout southwestern Japan. AKAIKA is distributed in the Ryukyu Islands and also probably occurs on the Pacific coast of Honshu, mainland Japan. KUAIKA is limited to the tropical region and its latitudinal distribution suggests a close correlation with water temperature. In SHIROIKA and KUAIKA, significant genetic differences were detected between the specimens from Ogasawara Islands and those from the other localities suggesting the existence of a certain barrier of panmixia between insular localities.

The oval squid, *Sepioteuthis lessoniana* (Lesson, 1830), is a loliginid squid widely distributed throughout the Indian Ocean and the western to central Pacific Ocean (Adam, 1939; Okutani, 1973). In Japan, this squid occurs in inshore waters extending from southern Hokkaido to Okinawa Islands (Sasaki, 1929; Okutani, 1973) and is one of the commercially important squids for neritic fisheries, especially in southwestern Japan (Dotsu *et al.*, 1981; Tsuchiya, 1982; Suzuki *et al*, 1983; Ueta *et al.*, 1992).

On Ishigaki Island, Okinawa, southwestern Japan, fishermen have traditionally distinguished the oval squid into three different groups, namely, AKAIKA, SHIROIKA, and KUAIKA, based on the size, color in freshly-killed condition, and the fishing season and ground (Okutani, 1984; Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994). Izuka *et al.* (1994) carried out allozyme electrophoresis resolving 11 loci in three groups of squids from Ishigaki Island. The results showed that the three groups differed from each other at least among three genetic loci. In addition, three types of egg capsules containing a different number of eggs per capsule were reported from Ishigaki Island (Segawa *et al.*, 1993a, b). Izuka *et al.* (1994) made it clear by allozyme electrophoresis that each group of squid produces different egg capsules: SHIROIKA produces egg capsules containing 4-8 eggs (mode = 6); KUAIKA lays two-egg capsules; and AKAIKA lays egg capsules containing 5-13 eggs (mean = 9.2; SD = 1.2). Further, each type of egg capsule is laid on a different substratum (Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994). These facts indicate that these three groups of *Sepioteuthis lessoniana* along the coast of Ishigaki Island are genetically and reproductively independent despite sympatry. Izuka *et al.* (1994) concluded that these three groups should be regarded as distinct species rather than as infraspecific fractions of a single species, *S. lessoniana.*

To date, allozyme electrophoresis has been a good tool for elucidating interspecific relationships (Augustyn and Grant, 1988; Brierley and Thorpe, 1994; Yokawa, 1994) and cryptic species (Smith *et al.*, 1981; Brierley *et al.*, 1993a; Yeatman and Benzie, 1993; Izuka *et al.*, 1994), and also providing useful information on population structure (*e.g.* Ally and Keck, 1978; Christofferson *et al.*, 1978; Garthwaite *et al.*, 1989; Brierley *et al.*, 1993b). The present study attempts to clarify the population structure and distribution of the three taxa currently referred to the *Sepioteuthis lessoniana* complex in southwestern Japan by allozyme analysis.

MATERIALS AND METHODS

A total of 554 specimens of *Sepioteuthis* were collected from ten localities in inshore waters of southwestern Japan and a single site at Rayong in the Gulf of Thailand by

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Table 1. Sampling data for squids used in the present study. Abbreviation of locality as mentioned in tables and figures are in parentheses.

Sampling Locality	Abbreviated Locality Name	N	Date of Collection
Ishigaki Island, Okinawa Pref.	(Ishigaki)	83	May-Oct. 1992
Amami Island, Kagoshima Pref.	(Amami)	22	Feb.-Mar. 1994
Ogasawara Islands, Tokyo	(Ogasawara)	178	June-Oct. 1994
Miyazu City, Kyoto	(Kyoto)	39	Oct. 1992
Sakai, Toyama Pref.	(Toyama)	17	Oct. 1992
Tsuruga, Fukui Pref.	(Fukui)	20	Oct. 1993
Nagato, Yamaguchi Pref.	(Yamaguchi)	36	Feb. 1993
Miura Peninsula, Sagami Bay	(Sagami)	8	July 1994
Hiwasa, Tokushima Pref.	(Tokushima)	102	Aug.-Dec. 1994
Shima, Mie Pref.	(Mie)	45	Aug. 1994
Rayong, Gulf of Thailand	(Thailand)	4	Jan. 1994
	Total	554	

set net, jigging, or scoop net during the period from May 1992 to December 1994 (Fig. 1, Table 1). The squid samples from Ishigaki Island used in the present study were the same as those studied by Izuka *et al.* (1994) with additional genetic data for three loci of *AAT-2**, *G3PDH**, and *PGM**. All the samples were transferred immediately after collection to the laboratory of Tokyo University of Fisheries and kept frozen at -80°C until analysis. Horizontal starch gel electrophoresis was carried out using buccal mass muscle (for details of methods, see Izuka *et al.*, 1994). Three buffer systems described by Numachi (1989) were used for electrode and gel: citrate-N-(3-aminopropyl) morpholine buffer at pH 6 and pH 7 (CAPM6 and CAPM7) and tris-citric acid buffer at pH 8 (CT-8N).

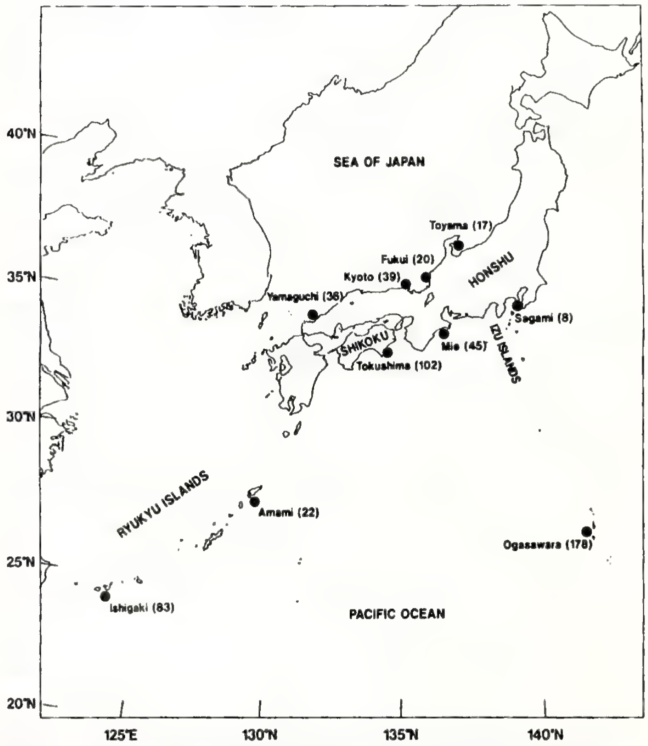

Fig. 1. Sampling localities of the specimens examined in the present study. Numbers in parentheses indicate number of specimens analyzed.

After electrophoresis, the gel was stained for ten enzymes (Table 2). Staining protocols were cited from Hillis and Moritz (1990) except for diaphorase which followed Harris and Hopkinson (1976). Nomenclature of locus and allele follows the guidelines of Shaklee *et al.* (1990). All the samples were classified into AKAIKA, SHIROIKA, and KUAIKA on the basis of asparate aminotransferase genotypes which are taxon-specific (Izuka *et al.*, 1994).

Differences in allele frequencies among localities within every combination of taxa were tested for significance ($P < 0.05$) using the chi square test (Kimura, 1960). Intrapopulational variability was evaluated by the proportion of polymorphic loci (P) (5% level) and heterozygosity (H). However, heterozygosity was not analyzed when the sample size was < 15 specimens for statistical reasons (Nei, 1987). Nei's (1972) genetic distance (D) was calculated from the data of allele frequencies, and the unweighted paired group method of cluster analysis (Sokal and Sneath, 1963) was employed to establish genetic relationships among the squids sampled.

RESULTS

We detected 47 individuals of AKAIKA from three localities, 470 SHIROIKA from 11 localities, and 37 KUAIKA from three localities (Fig. 2). No other kinds of oval squid were found in the present materials. The three taxa could be completely identified by fixed genetic differences at *AAT-1** (Table 3). Taxon-specific alleles were also recognized at *DIA** and *IDHP** in SHIROIKA, and at

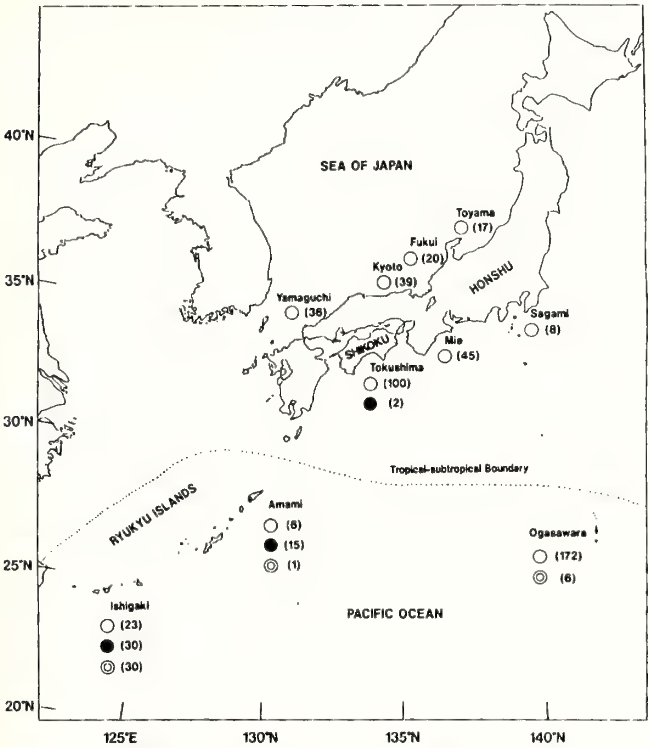

Fig. 2 . Distribution of each of the three taxa as detected by electrophoresis. ○, SHIROIKA ; ●, AKAIKA ; ◎, KUAIKA. Numbers in parentheses indicate number of specimens detected.

*MDH-1** and *SORD** in KUAIKA (Table 3).

AKAIKA

AKAIKA squid were found in samples from Tokushima, Shikoku, and Amami and Ishigaki Islands (Fig. 2). No significant difference in allele frequency was detected among these squids, and genetic distances among these sites were close to zero (Fig. 3). All loci were monomorphic except for a single *PGDH** heterozygote from Ishigaki Island (Table 3). These observations indicate that the squids of these three localities are not differentiated from each other and may share a common gene pool.

KUAIKA

KUAIKA was detected from the Ryukyu and Ogasawara Islands, and never observed in the seven samples from inshore waters around Honshu (Fig. 2). No significant genetic difference was detected between the specimens from Amami and Ishigaki Islands indicating that the KUAIKA squid population within the Ryukyu Islands is genetically uniform. However, a significant difference in allele frequency was detected by chi square test at *PGM** between the squids of Ryukyu Islands and Ogasawara Islands. The Ogasawara Islands specimens were fixed for *PGM*a* while only a few heterozygotes from Ishigaki Island exhibited this allele (Table 3). Genetic distance between the populations of Ogasawara Islands and those of Ryukyu Islands was 0.0689 (Fig. 3). Genetic variation was observed only in the squids of Ishigaki Island (Table 3) but the other samples are too small for comparison.

SHIROIKA

SHIROIKA squid were widely distributed through inshore waters around Honshu and from the Ogasawara and Ryukyu Islands, and in the Gulf of Thailand (Fig. 2). Except for the Ogasawara Islands sample, no significant genetic differences were observed among these populations and genetic distances were close to zero (Fig. 3). These facts suggest that SHIROIKA squid from Honshu, Shikoku, Ryukyu Islands, and Thailand could share common gene pool over their 2000 km geographical range. In contrast, slight but significant differences of allele frequencies were detected between the squids of Ogasawara Islands and those

Table 2. List of enzymes, loci examined, and buffers used for electrophoresis.

Enzyme	E.C. No.	Abbreviation	Loci	Buffer
Asparate aminotransferase	2.6.1.1	AAT	*AAT-1**	CAPM6
			*AAT-2**	"
Diaphorase	1.6	DIA	*DIA**	"
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	*G3PDH**	CAPM7
Glucose-6-phosphate isomerase	5.3.1.9	GPI	*GPI**	CAPM6
Isocitrate dehydrogenase	1.1.1.42	IDH	*IDH**	CAPM7
Malate dehydrogenase	1.1.1.37	MDH	*MDH-1**	"
			*MDH-2**	"
			*MDH-3**	"
Mannose-6-phosphate isomerase	5.3.1.8	MPI	*MPI**	CT-8N
6-Phosphogluconate dehydrogenase	1.1.1.44	PGDH	*PGDH**	CAPM7
Phosphoglucomutase	5.4.2.2	PGM	*PGM**	CAPM6
Sorbitol dehydrogenase	1.1.1.14	SORD	*SORD**	CAPM7

Table 3. Allele frequency, proportion of loci polymorphic (P), and heterozygosity (H) at 13 genetic loci by locality for three groups.

		AKAIKA			KUAIKA			SHIROIKA										
Locus	Allele	Ishigaki	Amami	Tokushima	Ishigaki	Amami	Ogasawara	Ishigaki	Amami	Ogasawara	Kyoto	Toyama	Fukui	Yamaguchi	Sagmi	Tokushima	Mie	Thailand
*AAT-1**	**a*	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	**b*	–	–	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	**c*	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(22)	(6)	(171)	(39)	(17)	(18)	(31)	(8)	(100)	(45)	(4)
*AAT-2**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(7)	(13)	(2)	(22)	(1)	(3)	(4)	(6)	(79)	(38)	(4)	(1)	(2)	(8)	(93)	(45)	(4)
*DIA**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	**b*	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(172)	(39)	(17)	(19)	(22)	(8)	(100)	(45)	(4)
*G3PDH**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(3)	(15)	(2)	(8)	(1)	(6)	(4)	(6)	(172)	(38)	(17)	(17)	(28)	(8)	(100)	(45)	(4)
*GPI**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.99	1.00	1.00
	**b*	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	0.01	–	–
	(n)	(15)	(15)	(2)	(30)	(1)	(6)	(17)	(6)	(171)	(37)	(6)	(18)	(21)	(8)	(100)	(45)	(4)
*IDHP**	**a*	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	**b*	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)
*MDH-1**	**a*	–	–	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	**b*	1.00	1.00	1.00	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)
*MDH-2**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(18)	(6)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)
*MDH-3**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(25)	(15)	(2)	(30)	(1)	(6)	(12)	(6)	(121)	(39)	(17)	(20)	(35)	(8)	(100)	(45)	(4)
*MPI**	**a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(4)	(23)	(6)	(163)	(39)	(17)	(19)	(36)	(8)	(100)	(45)	(4)
*PGDH**	**a*	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.01	0.03	–
	**b*	0.02	–	–	–	–	–	0.06	–	0.53	0.14	0.12	0.13	0.12	–	0.05	0.07	–
	**c*	0.98	1.00	1.00	1.00	1.00	1.00	0.93	1.00	0.47	0.82	0.79	0.83	0.86	0.94	0.89	0.82	1.00
	**d*	–	–	–	–	–	–	–	–	–	–	0.06	–	–	–	0.04	0.06	–
	**e*	–	–	–	–	–	–	–	–	–	0.04	0.03	0.02	0.02	0.06	0.01	0.02	–
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(171)	(39)	(17)	(20)	(3)	(8)	(100)	(45)	(4)
*PGM**	**a*	–	–	–	0.15	–	1.00	–	–	–	–	–	–	–	–	–	0.01	–
	**b*	1.00	1.00	1.00	0.85	1.00	–	1.00	0.92	1.00	0.99	1.00	0.90	0.97	1.00	0.94	0.94	1.00
	**c*	–	–	–	–	–	–	–	0.08	–	0.01	–	0.10	0.03	–	0.05	0.04	–
	(n)	(24)	(15)	(2)	(20)	(1)	(6)	(21)	(6)	(172)	(38)	(17)	(19)	(32)	(8)	(100)	(45)	(4)
*SORD**	**a*	–	–	–	–	–	–	–	–	–	0.05	0.06	–	0.02	–	0.03	0.03	–
	**b*	–	–	–	–	–	–	–	–	0.03	0.04	0.06	0.06	0.03	–	0.04	0.04	–
	**c*	–	–	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	**d*	1.00	1.00	1.00	–	–	–	1.00	1.00	0.96	0.91	0.87	0.94	0.95	1.00	0.92	0.92	1.00
	(n)	(22)	(15)	(2)	(30)	(1)	(6)	(16)	(6)	(172)	(39)	(16)	(18)	(29)	(8)	(100)	(45)	(4)
P		0.00	0.00	–	0.08	–	–	0.08	–	0.08	0.15	0.15	0.23	0.15	–	0.23	0.23	–
H		0.00	0.00	–	0.02	–	–	0.01	–	0.01	0.03	0.05	0.04	0.02	–	0.03	0.04	–

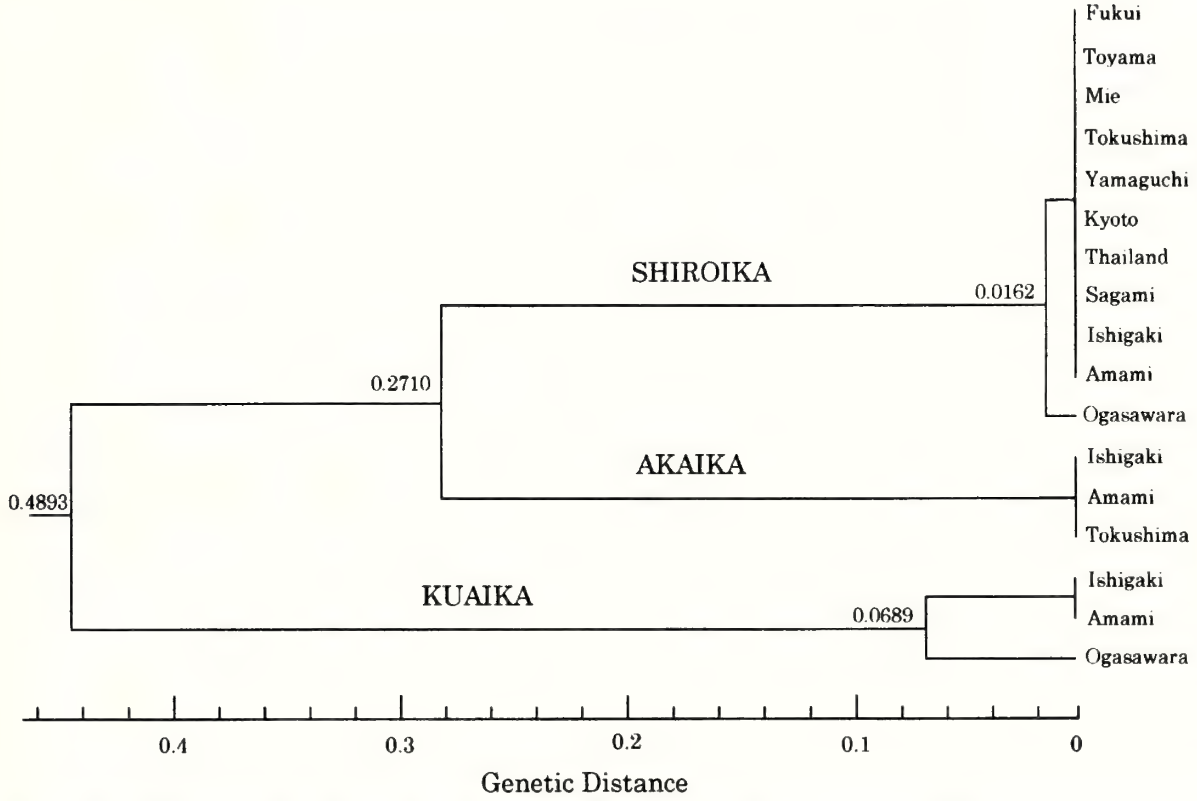

Fig. 3. Biochemical similarity dendrogram based on genetic distance among the squids by locality (for details on localities, see Table 1 and Fig. 1).

of the other localities (D = 0.0162) (Fig. 3).

Genetic variation was detected in all Japanese samples large enough to be tested. Mean heterozygosity (H) of the squids around Honshu was 0.037 (0.023-0.046) and the proportions of polymorphic loci (P) was 0.193 (0.154-0.231) (Table 3). Samples from Ishigaki Island and the Ogasawara Islands were slightly less variable (Table 3).

DISCUSSION

The present study clarified the distributional pattern of each taxon in the *Sepioteuthis lessoniana* complex around southwestern Japan. SHIROIKA is the common widely distributed squid in the tropical to warm temperate region of northwestern Pacific. AKAIKA was detected from Ryukyu Islands and the Pacific coast of Shikoku but probably extends easterly to the Izu Islands as egg capsules containing 6-12 eggs have been observed on deeper bottoms (40-50 m) there (Izuka, unpubl.). KUAIKA was the only taxon restricted to the Ryukyu and Ogasawara Islands and was never found around the main Japanese islands. Amami and Ogasawara Islands are both located in the northernmost part of the tropical region (Briggs, 1974; Nishimura, 1992) and lie near the isotherm of minimum winter temperature of about 20°C (*e. g.* Briggs, 1974; Dall, 1991). Izuka *et al.* (1994) assumed that KUAIKA were confined to the tropical western Pacific, because their egg capsules have been observed only on shallow coral reefs in Ishigaki Island, Okinawa Island, Palau Island, and New Guinea. These facts suggest that KUAIKA is a tropical squid which extends north to the tropical-subtropical boundary (Fig. 3).

Genetic differences appeared in allele frequencies of both SHIROIKA and KUAIKA between Ogasawara Islands and the other sampling localities. This fact may be evidence of a certain barrier against panmixia between these localities. Brierley *et al.* (1993b) found that populations of *Loligo forbesi* Steenstrup, 1856, from the British Isles and the Azores could be considered to be existing in allopatry because of large distance, oceanic depths, and ocean currents between sites. It is unlikely that SHIROIKA and KUAIKA in the Ogasawara Islands maintain sufficient gene flow with those of the mainland and Ryukyu Islands, as the populations of these two areas are separated (more than 1000 km) by the Kuroshio Current. If dispersal of

KUAIKA had taken place between these areas, allele **b* which was recognized as the common allele at *PGM** in the Ryukyu Islands should be detected in the Ogasawara Islands population (Table 3). It suggested that KUAIKA in the Ogasawara Islands is almost genetically segregated from the Ryukyu Islands.

The present study revealed that each of the three taxa of the *Sepioteuthis lessoniana* complex has a different distributional pattern. Within two of these taxa, the populations from the Ogasawara Islands are genetically a little different from those of the other areas sampled. However, the sampling sites in the present study were restricted on the northernmost rim of the western central Pacific. More work including investigations at more southern localities is now required to estimate interpopulational variabilities over the entire geographical range of each of the three taxa.

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LITERATURE CITED

Adam, W. 1939. Cephalopoda. Partie 1. Le genre *Sepioteuthis* Blainville, 1924. *Siboga Expedition* 55a:1-33, Leiden.

Ally, J. R. and S. A. Keck. 1978. A biochemical-genetic population structure study of market squid, *Loligo opalescens*, along the California coast. *Fish Bulletin, California* 169:113-121.

Augustyn, C. J. and W. S. Grant. 1988. Biochemical and morphological systematics of *Loligo vulgaris vulgaris* Lamarck and *Loligo reynaudi* d'Orbigny nov. comb. (Cephalopoda: Myopsida). *Malacologia* 29:215-233.

Brierley, A. S. and J. P. Thorpe. 1994. Biochemical genetic evidence supporting the taxonomic separation of *Loligo gahi* from the genus *Loligo*. *Antarctic Science* 6 (2):143-148.

Brierley, A. S., P. G. Rodhouse, J. P. Thorpe, and M. R. Clarke. 1993a. Genetic evidence of population heterogeneity and cryptic speciation in the ommastrephid squid *Martialia hyadesi* from the Patagonian Self and Antarctic Polar Frontal Zone. *Marine Biology* 116:593-602.

Brierley, A. S., J. P. Thorpe, M. R. Clarke, and H. R. Martins. 1993b. A preliminary biochemical genetic investigation of the population structure of *Loligo forbesi* Steenstrup, 1856 from the British Isles and the Azores. *In: Recent Advances in Cephalopod Fisheries Biology*, T. Okutani, R. K. O'Dor, and T. Kubodera, eds. pp. 59-67. Tokai University Press, Tokyo.

Briggs, J. C. 1974. *Marine Zoogeography.* McGraw-Hill, New York. 475 pp.

Christofferson, J. P., A. Foss, W. E. Lambert, and B. Welge. 1978. An electrophoretic study of select proteins from the market squid, *Loligo opalescens* Berry. *Fish Bulletin, California* 169:123-133.

Dall, W. 1991. Zoogeography of the Penaeidae. *Memoirs of the Queensland Museum* 31:39-50.

Dotsu, K., Y. Shimao, and Y. Natukari. 1981. [Ecology and fisheries of the oval squid, *Sepioteuthis lessoniana* Lesson in Goto Island.] *In: Goto-no-Seibutu (Animals and Plants in Goto Island),* Biological Society of Nagasaki Prefecture, ed. pp. 457-465. Nagasaki. (In Japanese).

Garthwaite, R. L., C. J. Barg Jr., and J. Harrigan. 1989. Population genetics of the common squid *Loligo pealei* LeSueur, 1821, from Cape Cod to Cape Hatteras. *Biological Bulletin* 177:287-294.

Harris, H. and D. A. Hopkinson. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics.* American Elsevier Publication Co. Inc., New York. [Looseleaf with supplements in 1977 and 1978].

Hillis, D. M. and C. Moritz. 1990. *Molecular Systematics.* Sinauer Associates, Sunderland, Massachusetts. 588 pp.

Izuka, T., S. Segawa, T. Okutani, and K. Numachi. 1994. Evidence on the existence of three species in the oval squid *Sepioteuthis lessoniana* complex in Ishigaki Island, Okinawa, southwestern Japan, by isozyme analyses. *Venus* 53 (3):217-228.

Kimura, M. 1960. *[An Introduction to Population Genetics.]* Baifukan, Tokyo. 312 pp. (In Japanese).

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106:283-292.

Nei, M. 1987. *Molecular Evolutionary Genetics.* Columbia University Press, New York. 512 pp.

Nishimura, S., ed. 1992. *[Guide to Seashore Animals of Japan with Color Pictures and Keys, Vol. I.]* Hoikusha Publishing Company, Ltd., Tokyo. 425 pp. (In Japanese).

Numachi, K. 1989. [Population differentiation of marine organisms by isozyme analysis.] *In: [Report on the Genetic Assessment Project],* Japan Fisheries Conservation Association, ed. Japan Fisheries Resources Conservation Association, Tokyo. (In Japanese).

Okutani, T. 1973. [Guide and keys to squid in Japan.] *Bulletin of Tokai Regional Fisheries Research Laboratory* 74:83-11. (In Japanese with English abstract).

Okutani, T. 1984. [Life history of oval squid, *Sepioteuthis lessoniana.*] *Saibai Giken* 13 :69-75. (In Japanese).

Sasaki, M. 1929. A monograph of the dibranchiate cephalopods of Japan and adjacent water. *Journal of Faculty of Agriculture, Hokkaido Imperial University,* Supplement 20:1-357, 35 pls.

Segawa, S., S. Hirayama, and T. Okutani. 1993a. Is *Sepioteuthis lessoniana* in Okinawa a single species? *In: Recent Advances in Cephalopod Fisheries Biology,* T. Okutani, R. K. O'Dor, and T. Kubodera, eds. pp. 513-521. Tokai University Press, Tokyo.

Segawa, S., T. Izuka, T. Tamashiro, and T. Okutani. 1993b. A note on mating and egg deposition by *Sepioteuthis lessoniana* in Ishigaki Island, Okinawa, southwestern Japan. *Venus* 52 :101-108.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. *Transactions* *of the American Fisheries Society* 119 :2-15.

Smith, P. J., P. E. Roberts, and R. J. Hurst. 1981. Evidence for two species of arrow squid in the New Zealand fishery. *New Zealand Journal of Marine Freshwater Research* 15:247-253.

Sokal, R. R. and P. H. A. Sneath. 1963. *Principles of Numerical Taxonomy*. Freeman, San Francisco. 359 pp.

Suzuki, S., A. Kuwahara, and K. Washio. 1983. [Study on the fishing conditions and ecological aspect of squid, *Loligo edulis budo* and *Sepioteuthis lessoniana* in the coastal waters off Kyoto Prefecture.] *Bulletin of Japanese Society of Fishery Oceanography* 42:21-27 (In Japanese with English abstract).

Tsuchiya, M. 1982. [On the culture of the squid, *Sepioteuthis lessoniana* Lesson (Loliginidae), from hatchling to the whole life in Iriomote Island, Okinawa.] *Bulletin of Institute of Oceanic Research and Development, Tokai University* 4:49-70. (In Japanese with English abstract).

Ueta, Y., S. Segawa, M. Tenjin, Y. Jo, I. Kitakado, M. Fukunaga, and Y. Kangawa. 1992. [Occurrence and character of egg masses and estimation of egg-laying and hatchling period on squid, *Sepioteuthis lessoniana* in outer water adjacent to Kii Channel.] *Suisanzoshoku* 40:469-474. (In Japanese with English abstract).

Yeatman, J. and J. A. H. Benzie. 1993. Cryptic speciation in *Loligo* from northern Australia. *In: Recent Advances in Cephalopod Fisheries Biology,* T. Okutani, R. K. O'Dor, and T. Kubodera, eds. pp. 17-26, Tokai University Press, Tokyo.

Yokawa, K. 1994. Allozyme differentiation of sixteen species of ommastrephid squid (Mollusca, Cephalopoda). *Antarctic Science* 6(2):201-204.

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Ctenopteryx sicula, a bathypelagic loliginid squid?

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Abstract: *Ctenopteryx sicula* (Vérany, 1851) is an open-eyed, deep-water squid, and as such has traditionally been classified in the suborder Oegopsida (family Ctenopterygidae) along with other families of squid exhibiting these characteristics. *C. sicula* however displays numerous morphological features, including fused axons in the giant nerve fiber system and accessory nidamental glands, found otherwise only within members of the myopsid families Loliginidae and Pickfordiateuthidae. This has lead previous authors to suggest that *Ctenopteryx* species would be more appropriately placed in the suborder Myopsida. Here biochemical genetic evidence is presented which indicates that *C. sicula* is more closely related to several loliginid species than to species of the oegopsid families Histioteuthidae, Ommastrephidae, and Enoploteuthidae. These data, in conjunction with new data on comparative beak morphology, also suggest that *C. sicula* should be considered an oceanic myopsid species.

The squids (Teuthoidea) are generally considered to be divided into two suborders: the Oegopsida which are mainly oceanic, open-water species which lack a corneal membrance covering the eye, and the Myopsida which are mainly near-shore species and which posses a corneal membrane. This membrane has been described as an adaptation preventing ocular damage in shallow, sediment-rich waters of continental shelf areas (Morton and Yonge, 1964). *Ctenopteryx sicula* (Vérany, 1851) (family Ctenopterygidae) is a bathypelagic squid species of cosmopolitan oceanic distribution (Nesis, 1987; Roeleveld *et al.*, 1992). The species lacks an eye-covering corneal membrane and as such is classified in the suborder Oegopsida along with other families of squid exhibiting this characteristic (for example, see Roper *et al.*, 1969; Nesis, 1987). Naef (1923) and Young (1991) have however highlighted a number of morpological features which are at odds with this scheme of classification. *C. sicula* exhibits fusion of nerve axons in the giant fiber system, and has accessory nidamental glands, features which are otherwise found exclusively within the myopsid families Loliginidae and Pickfordiateuthidae. In addition, *C. sicula* has attachments of the fourth buccal connectives similar to those of *Loligo* species, and has suckers on the buccal lappets, exhibits retraction of tentacles into pockets, and has straight, simple funnel locking cartilages, all of which are reminiscent of loliginid morphology (Naef, 1923; Nesis, 1987; Young, 1991). Young (1991) consequently suggested that *C. sicula* could be a squid species related to the loliginids which has become adapted to life in deep water. Here biochemical genetic techniques are used to investigate relationships between *C. sicula* and member species of the families Loliginidae, Histioteuthidae, Ommastrephidae, and Enoploteuthidae in an attempt to determine to which families it is most closely related, and hence with which suborder the species has closet affinity.

Enzyme electrophoresis is well established as a tool for investigation of taxonomy and systematics (Avise, 1974, 1983; Ferguson, 1980; Ayala, 1983; Richardson *et al.*, 1986; Thorpe and Solé-Cava, 1994), and has been applied to a number of cephalopod problems (Smith *et al.*, 1981; Augustyn and Grant, 1988; Brierley and Thorpe, 1994; Yokowa, 1994; Brierley *et al.*, in press). The theoretical basis for the use of electrophoretic data in taxonomic studies is founded on what has become known as the *molecular clock hypothesis* (Thorpe, 1982; Nei,1987). This hypothesis holds that between reproductively isolated groups of organisms molecules such as enzymes, the structures of which are under direct genetic control, diverge at a rate that is stochastically related to the evolutionary time since divergence. The majority of cases employing electrophoresis as a taxonomic aid have addressed questions raised at the level of populations, species, or genera. The technique has however also provided meaningful data for comparisons between species from related families (for example, see Solé-Cava *et al.*, 1992, 1994), and is therefore an appropriate means for investigation of the question in hand.

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MATERIALS AND METHODS

Squid samples

Ctenopteryx sicula (Vérany, 1851) and the oegopsid squid species *Pyroteuthis margaritifera* (Ruppell, 1844), *Histioteuthis bonellii* (Férussac, 1835), and *Todarodes sagittatus* (Lamarck, 1799) were caught from the northeastern Atlantic Ocean in the region of 21°W, 31°N using a multiple-opening, 8 m^2 rectangular mid-water trawl (RMT8) during RRS *Discovery* cruise 194 in August 1990 (see Herring, 1990). *Sepioteuthis lessoniana* Lesson, 1830, was obtained from Indonesian waters, and *Loligo forbesi* Steenstrup, 1856, and *Loligo vulgaris vulgaris* Lamarck, 1798, were caught around the British Isles. Samples of mantle tissue were dissected from each specimen as soon after capture as possible, and immediately frozen (to -80°C in the case of the *Discovery* samples) prior to transportation to Port Erin Marine Laboratory. Date and location of capture, and species sample sizes, are presented in Table 1.

Electrophoresis

Mantle tissue samples from *Ctenopteryx sicula* and the other oegopsid species were assayed during September 1990 using a series of standard electrophoretic techniques which have been described in detail elsewhere (Brierley, 1992; Brierley *et al.*, 1993a, b). The majority of the loliginids had been examined previously using the same experimental protocols, but a group of ten were run again concurrently with the *Discovery* samples in order to provide a reference for relative band migration. Gels were scored immediately after optimum stain development, and genotypes assigned accordingly.

Data analysis

Allele frequency data was analyzed using the FORTRAN program BIOSYS-1 (Release 1.7) (Swofford and Selander, 1981) to calculate unbiased genetic identity *(I)* and distance *(D)* (Nei, 1978) between species pairs, and to construct a dendrogram of *D* using the unweighted pair-grouping arithmetic mean (UPGMA) cluster analysis algorithm (Sneath and Sokal, 1973). The use of the UPGMA method in conjunction with *D* is generally recognized as the best and most accurate procedure for phylogentic tree reconstruction from electrophoretic data (Nei, 1987). This is partly because *D* can be subject to large stochastic errors (especially if calculated with small numbers of loci), and the procedure of distance-averaging used in UPGMA reduces this error considerably (Nei, 1987).

RESULTS

Seventeen enzyme stain systems developed successfully for all species, revealing the presence of 18 common putative enzyme loci. The group of ten loliginids run concurrently with *Ctenopteryx sicula* and the oegopsid species resolved identically here as previously (Brierley *et al.*, 1993a, 1995; Brierley and Thorpe, 1994). A slight reduction in band intensity was apparent, but band migration and definition remained entirely unaffected by frozen (-26°C) storage. Allele frequencies at the 18 clearly resolving enzyme loci are given for each species in Table 2. Inspection of Table 2 reveals *Histioteuthis bonellii, Todarodes sagittatus*, and *Pyroteuthis margaritifera* exhibiting very low levels of mean heterozygosity per locus, a phenomemon which is a seemingly recurrent theme in studies of squid biochemical genetics (see review in Brierley *et al.*, 1993a). Although this ubiquitous feature of low intraspecific genetic variability can cause problems for the use of electrophoretic data in squid population studies (see Brierley *et al.*, 1993b, 1995), taxonomic studies benefit because banding patterns remain simple, aiding interpretation and greatly reducing the likelihood of error (Garthwaite *et al.*, 1989). Mean *I* and *D* between all species pairs are given in Table 3. These unbiased estimates are most appropriate for small sample sizes, especially, as here, when observed heterozygosity levels are low. *D* is purported to be stochastically linear with evolutionary time (Nei, 1987; Thorpe, 1989), and varies between a theoretical minimum of zero in comparison of genetically identical individ-

Table 1. Sample size, and date and location of capture of species examined.

Species	Sample size	Date of capture	Location of capture
Ctenopteryx sicula	4	August 1990	Northeastern Atlantic Ocean
Pyroteuthis margaritifera	11	August 1990	Northeastern Atlantic Ocean
Histioteuthis bonellii	14	August 1990	Northeastern Atlantic Ocean
Todarodes sagittatus	2	August 1990	Northeastern Atlatnic Ocean
Sepioteuthis lessoniana	5	April 1988	South Alas Strait, Indonesia
Loligo forbesi	20	October 1989	Isle of Man, British Isles
L. vulgaris vulgaris	20	October 1989	Plymouth, United Kingdom

Table 2. Allele frequencies at the 18 clearly resolving enzyme loci.

Locus	Allele	*Ctenopteryx sicula* N = 4	*Loligo forbesi* N = 20	*L. v. vulgaris* N = 20	*Sepioteuthis lessoniana* N = 5	*Pyroteuthis margaritifera* N = 11	*Histioteuthis bonnellii* N = 14	*Todarodes sagittatus* N = 2
aGpdh	A	0	0	0	0	0	1	0
	B	0.125	0	0	0	0	0	0
	C	0.875	0	0	0	0	0	1
	D	0	0	0.9	0	0	0	0
	E	0	1	0.1	0	0	0	0
	F	0	0	0	1	1	0	0
Mdh- 1	A	0	1	1	0	0	0	0
	B	0	0	0	1	0	0	0
	C	0.875	0	0	0	0	0	0
	D	0	0	0	0	1	0	1
	E	0.125	0	0	0	0	1	0
Mdh-2	A	0	0	0	0	0	1	0
	B	1	0	0	1	0	0	0
	C	0	1	1	0	0	0	0
	D	0	0	0	0	0	0	1
	E	0	0	0	0	1	0	0
Me	A	0	0	0	1	0	0	0
	B	0	1	1	0	0	0	0
	C	0	0	0	0	1	0	0
	D	1	0	0	0	0	0	0
	E	0	0	0	0	0	1	0
	F	0	0	0	0	0	0	1
Idh	A	0	0	0	0	0	0	1
	B	0	0	0	0	0	1	0
	C	1	0	0	1	0	0	0
	D	0	0.95	1	0	0	0	0
	E	0	0.05	0	0	1	0	0
Pgdh	A	0	0	0	1	0	0	0
	B	0	0	1	0	0	0	0
	C	0	0.025	0	0	0	0	0
	D	0	0.975	0	0	0	0	0
	E	0	0	0	0	1	0	0
	F	1	0	0	0	0	0	0
	G	0	0	0	0	0	1	0
	H	0	0	0	0	0	0	1
Gopdh	A	0	0	0	0	1	0	0
	B	0	0	0	0	0	0	1
	C	0	1	0	1	0	0	0
	D	0	0	1	0	0	0	0
	E	0	0	0	0	0	1	0
	F	1	0	0	0	0	0	0
Gapdh	A	0	0	0	0	1	0	0
	B	0	0	0	0	0	0	1
	C	0	1	0	0	0	0	0
	D	0	0	0	1	0	0	0
	E	1	0	1	0	0	0	0
	F	0	0	0	0	0	1	0
Sdh	A	0	0	0	0	0	1	0
	B	0	0	0	0	1	0	0
	C	1	0	0	0	0	0	0
	D	0	1	0	0	0	0	0
	E	0	0	0	1	0	0	1
	F	0	0	1	0	0	0	0
Pep-A	A	1	0	0	0	0	0	0
	B	0	1	0	0	0	0	0
	C	0	0	1	0	0	0	0
	D	0	0	0	0	0	0	1
	E	0	0	0	1	0	0	0
	F	0	0	0	0	1	0	0
	G	0	0	0	0	0	1	0

(Continued)

Table 2. Continued.

Locus	Allele	*Ctenopteryx sicula* N = 4	*Loligo forbesi* N = 20	*L. v. vulgaris* N = 20	*Sepioteuthis lessoniana* N = 5	*Pyroteuthis margaritifera* N = 11	*Histioteuthis bonnellii* N = 14	*Todarodes sagittatus* N = 2
Ck	A	0.125	0	0	0	0	0	0
	B	0.875	0	0	0	0	0	0
	C	0	1	1	0	0	0	0
	D	0	0	0	1	0	0	0
	E	0	0	0	0	1	1	1
Fum	A	0	0	0	0	0	0	1
	B	0	1	0	0.5	0	0	0
	C	1	0	0	0	0	0	0
	D	0	0	0	0	1	0	0
	E	0	0	1	0	0	0	0
	F	0	0	0	0.5	0	0	0
	G	0	0	0	0	0	1	0
Mpi	A	0	0	0	0.5	0	0	0
	B	0	0	0	0	0	0	1
	C	0	0	0	0.5	0	0	0
	D	0	0	1	0	0	0	0
	E	1	0.975	0	0	0	0	0
	F	0	0.025	0	0	1	1	0
Gpi	A	0	0	0	0	0	0	1
	B	0	0	0	0	1	0	0
	C	1	0	0	0	0	0	0
	D	0	1	1	0	0	0	0
	E	0	0	0	1	0	0	0
	F	0	0	0	0	0	1	0
Ald	A	0	1	1	1	0	1	1
	B	1	0	0	0	0	0	0
	C	0	0	0	0	1	0	0
Odh	A	0	1	0	1	0	0	0
	B	1	0	1	0	1	0	0
	C	0	0	0	0	0	1	0
	D	0	0	0	0	0	0	1
Ldh	A	1	0	0	0	0	0	0
	B	0	1	0.825	0	0	0	0
	C	0	0	0	0	1	0	0
	D	0	0	0	1	0	0	0
	E	0	0	0.175	0	0	0	0
	F	0	0	0	0	0	1	0
	G	0	0	0	0	0	0	1
Sordh	A	0	0.125	0	0	0	0	0
	B	0	0.85	0	1	0	0	0
	C	0	0.025	0	0	0	0	0
	D	0	0	1	0	0	0	0
	E	1	0	0	0	0	0	0
	F	0	0	0	0	1	0	0
	G	0	0	0	0	0	0	1
	H	0	0	0	0	0	1	0

Table 3. Matrix of unbiased genetic identity *(I)* (above diagonal) and distance *(D)* (below diagonal) values between all species pairs.

Species	1	2	3	4	5	6	7
1 *Ctenopteryx sicula*	****	0.056	0.115	0.117	0.057	0.007	0.05
2 *Loligo forbesi*	2.881	****	0.449	0.253	0.004	0.058	0.056
3 *L. vulgaris vulgaris*	2.162	0.800	****	0.058	0.056	0.056	0.056
4 *Sepioteuthis lessoniana*	2.144	1.375	2.845	****	0.057	0.057	0.115
5 *Pyroteuthis margaritifera*	2.869	5.468	2.877	2.859	****	0.111	0.111
6 *Histioteuthis bonnellii*	4.949	2.853	2.877	2.859	2.197	****	0.111
7 *Todarodes sagittatus*	3.003	2.877	2.877	2.165	2.197	2.197	****

uals, and infinity when the pair of taxa under consideration exhibits no common genetic characters. A UPGMA dendogram of *D* (Fig. 1) shows *Ctenopteryx sicula* clustering more closely with the branch encompassing the loliginid species than to the branch containing the oegopsid families Histioteuthidae, Ommastrephidae, and Enoploteuthidae.

DISCUSSION

In contrast to electrophoretic studies of population structuring, in which large sample sizes are required, taxonomic studies such as the one reported here can be successfully conducted using only very small numbers of individuals from each taxa if large numbers of enzyme loci are studied (see for example Richardson *et al.*, 1986). The comparatively small sample sizes of *Ctenopteryx sicula, Todarodes sagittatus,* and *Sepioteuthis lessoniana* are unlikely to be a major source of inaccuracy in calculation of *D*, or, consequently, in reconstruction of the phylogenetic tree shown in Fig. 1. Numbers of animals used have negligible effects upon the errors of genetic distance, and even sample sizes as small as one will give acceptable distance estimates (see Nei, 1978; Gorman and Renzi, 1979; Thorpe, 1982). This fact is well illustrated by a reconstructed phylogeny of the family Ommastrephidae (Yokowa, 1994) which is based on electrophoretic data gathered from 16 species with a mean sample size of less than two.

Yeatman and Benzie (1993) used *Sepioteuthis lessoniana* as an outgroup in a study of cryptic speciation in *Loligo* from Australian waters. They reported the species differing completely from all *Loligo* taxa investigated at eight of the 11 loci they resolved, and consequently reported a *D* value of > 4 between species. Such a high value is far larger than would normally be expected between members of confamilial genera (Thorpe, 1982). We have assayed six (*Ak, Gpi, Idh, Mdh-1, Mdh-2,* and *Mpi*) of these eight loci here and elsewhere (Brierley, 1992; Brierley *et al.*, 1993b, in press) and similarly find alleles unique to *Sepiotheuthis lessoniana* at these loci. However this and our previous analyses have included data from a number of other loci in addition to the 11 investigated by Yeatman and Benzi (1993), and our estimate of *D* between *Loligo* species and *Sepioteuthis lessoniana* of the order of 1.4 is therefore likely to be the more accurate. This large discrepancy between studies highlights the possibility of substantial interlocus errors on measures of genetic distance (Thorpe, 1979, 1982), and emphasizes the importance of screening large numbers of loci.

The phylogenetic tree reconstructed using UPGMA analysis of *D* between all species pairs (Fig. 1) comprises two main branches, one containing member species of the suborder Oegopsida, and the other containing myopsid species. *Ctenopteryx sicula* clusters more closely with the Myopsida, and genetic data presented here are therefore in agreement with morphological evidence (Naef, 1923; Young, 1991) suggesting the species is related to *Loligo. C. sicula* clusters with other myopsid species at a level of *D* which lies within the practical limits of the measure. The *D* value of about 2.9 exhibited here between *C. sicula* and *Loligo forbesi* (see Table 3) is less than the value of *D* = 3, beyond which the measure diverges from linearity (Thorpe, 1989). This limit arises because *D* appears to suffer a "saturation" effect (Thorpe, 1982): a maximum of one nucleotide substitution per locus can be detected electrophoretically, and practical limitations impose a resolution threshold on

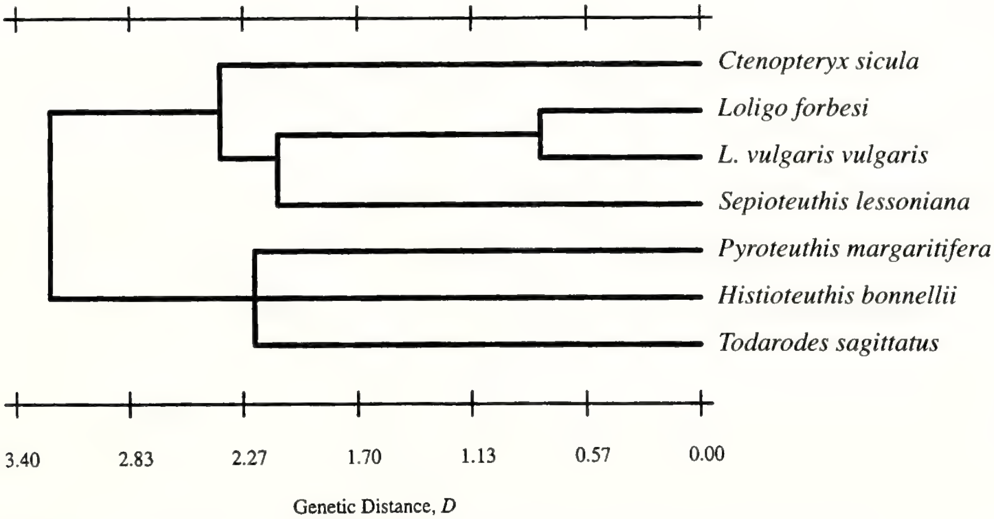

Fig. 1. UPGMA dendrogram of Nei's (1978) unbiased genetic distance *(D)* between all species pairs.

the number of discrete alleles which can be electrophoretically distinguished. Using data gathered from a survey of around 100 loci between species pairs from different major taxa, Thorpe (1982) found an approximate 5% coincidental identity between quite unrelated taxa (I in the order of 0.05, $D = -\log_e I$). The similarity between *C. sicula* and the loliginid species studied here is clearly greater than this 5% similarity. The level at which the oegospid and myopsid species cluster is high, but this does not detract from the validity of the observation that members of the Myopsida and Oegopsida cluster as two discrete entities, or that *C. sicula* is more closely related to loliginids than to any of the oegospid families studied here. We do not attempt to place an exact time of divergence on any of the branch points in Fig. 1, because the absolute relationship between genetic distance and evolutionary time is questionable (Sarich, 1977; Lessios, 1979; Thorpe, 1982; Smith and Coss, 1984). For most taxonomic purposes however, as here, the problem of absolute nonlinearity of D with time is unimportant because only relative values are needed (Thorpe, 1982). As long as genetic distance is approximately linear with time, the relative evolutionary relationships among organisms can be determined (Nei,1987).

Ctenopteryx sicula is positioned within the branch of the phylogenetic tree (Fig. 1) containing *Loligo forbesi* and *L. vulgaris vulgaris* beyond the junction at which *Sepioteuthis lessoniana* diverges from the genus *Loligo*. *Sepioteuthis* has been described, on morphological and distributional grounds, as a Tethyan relict genus which split off from all other loliginids before the closing of the Tethys Sea (Brakoniecki, 1986). *C. sicula* would therefore appear to have diverged from the loliginid line at some time before this event. Naef (1923) suggested that *C. sicula* should be considered the earliest independent branch of the stem of the higher Oegopsida. Genetic data from the present study summarized in the phenogram in Fig. 1 go some way to support this assertion, although further electrophoretic analysis of *Pickfordiateuthis pulchella* Voss, 1953, would be necessary before genetic and morphological data could be viewed in complete congruence. It would seem appropriate therefore to adopt the viewpoint of Young (1991), who has proposed that *C. sicula* may be considered as a teuthoid related to loliginids which has become adapted to a deep-water, oceanic life. Young (1991) has also argued that, despite the fact that the name is inappropriate for a squid exhibiting the open-eyed condition, it would be desirable to classify *Ctenopteryx* species with the families Loliginidae and Pickfordiateuthidae in the suborder Myopsida.

The numerous similarities between *Ctenopteryx* and *Bathyteuthis* species have been well described (Roper, 1969; Nesis, 1987), and more recent analyses of comparative beak morphology have further highlighted the affinity among *Loligo, Ctenopteryx,* and *Bathyteuthis* species (Roper and Clarke, unpub. data). It would be of additional interest to examine *Bathyteuthis* species electrophoretically, as it remains possible that these species similarly fail to comply with the broad categorization of an open-eyed, open-ocean suborder, contrasting entirely with a distantly related shelf-inhabiting group. Our data suggest that the character of the presence or absence of a corneal membrane may not be an infallible guide for distinguishing the two major systematic groupings of squid.

ACKNOWLEDGMENTS

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LITERATURE CITED

Augustyn, C. J. and W. S. Grant. 1988. Biochemical and morphological systematics of *Loligo vulgaris vulgaris* Lamarck and *Loligo vulgaris reynaudi* D'Orbigny nov. comb. (Cephalopoda: Myopsida). *Malacologia* 29:215-233.

Avise, J. C. 1974. Systematic value of electrophoretic data. *Systematic Zoology* 23:465-481.

Avise, J. C. 1983. Protein variation and phylogenetic reconstruction. *In: Protein Polymorphism: Adaptive and Taxonomic Significance,* G. S. Oxford and D. Rollinson, eds. pp. 103-130. Academic Press, London.

Ayala, F. J. 1983. Enzymes as taxonomic characters: *In: Protein Polymorphism: Adaptive and Taxonomic Significance,* G. S. Oxford and D. Rollinson, eds. pp. 3-26. Academic Press, London.

Brakoniecki, T. F. 1986. *A Generic Revision of the Family Loliginidae (Cephalopoda: Myopsida) Based Primarily on the Comparative Morphology of the Hectocotylus.* Doctoral dissertation, University of Miami, Miami, Florida. 163 pp.

Brierley, A. S. 1992. *Aspects of Genetic Diversity and Population Structure in Squid.* Doctoral dissertation, University of Liverpool. 154 pp.

Brierley, A. S. and J. P. Thorpe. 1994. Biochemical genetic evidence supporting the taxonomic separation of *Loligo gahi* from the genus *Loligo. Antarctic Science* 6(2):143-148.

Brierley, A. S., P. G. Rodhouse, J. P. Thorpe, and M. R. Clarke. 1993a. Genetic evidence of population heterogeneity and cryptic speciation in the ommastrephid squid *Martialia hyadesi* from the Patagonian Shelf and Antarctic polar frontal zone. *Marine Biology* 116(4):593-602.

Brierley, A. S., J. P. Thorpe, M. R. Clarke, and H. R. Martins. 1993b. A preliminary biochemical genetic investigation of the population structure of *Loligo forbesi* Steenstrup, 1856 from the British Isles and the Azores. *In: Recent Advances in Cephalopod Fisheries Biology,* T. Okutani, R. K. O'Dor, and T. Kubodera, eds. pp. 61-

69. University Press, Tokyo.

Brierley, A. S., J. P. Thorpe, G. J. Pierce, M. R. Clarke, and P. R. Boyle. 1995. Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the northeast Atlantic. *Marine Biology* 122:79-86.

Brierley, A. S., A. L. Allcock, J. P. Thorpe, and M. R. Clarke. In press. Biochemical genetic evidence supporting the taxonomic separation of *Loligo edulis* and *Loligo chinensis* (Cephalopoda: Teuthoidea) from the genus *Loligo*. *Marine Biology*.

Ferguson, A. 1980. *Biochemical Systematics and Evolution*. Blackie, Glasgow. 194 pp.

Garthwaite, R. L., C. J. Berg, Jr., and J. Harrigan. 1989. Population genetics of the common squid *Loligo pealei* Le Seur, 1821, from Cape Cod to Cape Hatteras. *Biological Bulletin* 177:287-294.

Gorman, G. C. and J. Renzi. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. *Copeia* 1979:361-364.

Herring, P. J. 1990. *RRS Discovery Cruise 194 04 Aug-02 Sep 1990. Comparative Benthic Biology at 31°N 20°W (Madeira Abyssal Plain) and Associated Midwater Studies*. Institute of Oceanographic Sciences Deacon Laboratory, Cruise Report 219. 57 pp.

Lessios, H. A. 1979. Use of Panamanian sea urchins to test the molecular clock. *Nature* 280:599-601.

Morton, J. E. and C. M. Yonge. 1964. Classification and structure of the Mollusca. *In: Physiology of Mollusca*, K. M. Wilbur and C. M. Yonge, eds. pp. 1-58. Academic Press, New York.

Naef, A. 1921-1923. *Fauna and Flora of the Bay of Naples. 35. Cephalopoda*. English translation by the Israel Program for Scientific Translation in 1972, Jerusalem. 917 pp.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York. 512 pp.

Nesis, K. N. 1987. *Cephalopods of the World*. T. F. H. Publications, Neptune City, New Jersey. 351 pp.

Richardson, B. J., P. R. Baverstock, and M. Adams. 1986. *Allozyme Electrophoresis - a Handbook for Animal Systematics and Population Studies*. Academic Press, Sydney. 410 pp.

Roeleveld, M. A. C., M. R. Lipinski, C. J. Augustyn, and B. A. Stewart. 1992. The distribution and abundance of cephalopods on the continental-slope of the eastern south-Atlantic. *South African Journal of Marine Science* 12:739-752.

Roper, C. F. E. 1969. Systematics and zoogeography of the worldwide bathypelagic squid *Bathyteuthis* (Cephalopoda, Oegopsida). *Bulletin of the United States National Museum* 291, 210 pp.

Roper, C. F. E., R. E. Young, and G. L. Voss. 1969. An illustrated key to the families of the order Teuthoidea (Cephalopoda). *Smithsonian Contributions to Zoology* 13, 32 pp.

Sarich, V. M. 1977. Rates, sample sizes and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature* 265:24-48.

Smith, D. G. and R. G. Coss. 1984. Calibrating the molecular clock: estimates of ground squirrel divergence made using fossil and geological time markers. *Molecular Biology and Evolution* 1:249-259.

Smith, P. J., P. E. Roberts, and R. J. Hurst. 1981. Evidence for two species of arrow squid in the New Zealand fishery. *New Zealand Journal of Marine and Freshwater Research* 15:247-253.

Sneath, P. H. A. and R. R. Sokal. 1973. *Numerical Taxonomy. The Principles and Practise of Numerical Classification*. Freeman, San Francisco, California. 573 pp.

Swofford, D. L.and R. B. Selander. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72:281-283.

Solé-Cava, A. M., N. Boury-Esnault, J. Vacelet, and J. P. Thorpe. 1992. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea. *Marine Biology* 113:299-304.

Solé-Cava, A. M., C. A. M. Russo, M. E. Araujo, and J. P. Thorpe. 1994. Cladistic and phenetic analysis of allozyme data for nine species of sea anemones of the family Actiniidae (Cnidaria: Anthozoa). *Biological Journal of the Linnean Society* 52(3):225-239.

Thorpe, J. P. 1979. Enzyme variation and taxonomy; the estimation of sampling errors in measurement of interspecific genetic similarity. *Biological Journal of the Linnean Society* 11:369-386.

Thorpe, J. P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics* 13:139-168.

Thorpe, J. P. 1989. Possible effects of interprotein variation in mean rate of amino acid substitution on the relationship of genetic distance with time since evolutionary divergence. *Biological Journal of the Linnean Society* 37:335-344.

Thorpe, J. P. and A. M. Solé-Cava. 1994. The use of electrophoresis in invertebrate systematics. *Zoological Scripta* 23(1):3-18.

Yeatman, J. and J. A. H. Benzie. 1993. Cryptic speciation in *Loligo* from northern Australia. *In: Recent Advances in Cephalopod Fisheries Biology*, T. Okutani, R. K. O'Dor, and T. Kubodera, eds., pp. 641-652. Tokai University Press, Tokyo.

Yokawa, K. 1994. Allozyme differentiation of sixteen species of ommastrephid squid (Mollusca, Cephalopoda). *Antarctic Science* 6(2):210-204.

Young, J. Z. 1991. *Ctenopteryx* the comb-fin squid is related to *Loligo*. *Bulletin of Marine Science* 49(1-2):148-161.

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Biogeography of *Octopus* species (Cephalopoda: Octopodidae) from southeastern Australia

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Abstract: Seven species of inshore, benthic octopuses have been recorded from temperate waters off southeastern Australia. Studies on aspects of reproductive biology show that five species (*Octopus berrima* Stranks and Norman, 1993; *O. bunurong* Stranks, 1990; *O. kaurna* Stranks, 1990; *O. pallidus* Hoyle, 1885; and *O. superciliosus* Quoy and Gaimard, 1832) produce large eggs (8-14 mm long) in low numbers (10s or 100s), and have hatchlings that immediately adopt a benthic existence. Another two species (*O. maorum* Hutton, 1880, and *O. warringa* Stranks, 1990) produce small to medium eggs (2-7 mm long) in high numbers (1000s), with juveniles that are temporarily planktonic before settling out to the benthos. The distribution of these species is influenced by regional oceanographic factors such as the Leeuwin Current, West Wind Drift, and East Australian Current. The five species with direct development have limited means for dispersal, and are restricted in distribution to waters off southeastern Australia; the other two species with indirect development have the potential for long-range dispersal through the waters off southeastern Australia, the Tasman Sea, and New Zealand.

The family Octopodidae is typically characterized by the inshore, benthic octopus that possesses a saccular mantle and eight arms. The best-documented genus in the family is *Octopus*, which has a distribution in all coastal regions except for Arctic and Antarctic areas (Nesis, 1987).

There is a rich and diverse octopodid fauna in tropical to cold-temperate waters of the Australasian continental shelf region (Lu and Dunning, in press; Stranks, in press). During the past decade, the systematics of the regional fauna have been reviewed and revised by several workers, for example, the tropical fauna of Australia by Norman (1992a, b; 1993a, b, c), the cold temperate fauna of Australia by Stranks (1988a, b; 1990) and Stranks and Norman (1993), and the fauna of New Zealand by O'Shea (1990). These studies recognized 19 valid species of *Octopus* from Australia, and eight species from New Zealand. In addition, these works established distributional ranges for most of the valid species, and reported data on general biology where available.

Wilson and Allen (1987) included a general summary of molluscan biogeography from Australia. Lu and Phillips (1985) and Lu and Dunning (in press) have summarized available information on the distribution and biogeography of Australian cephalopods, but there has been little information published on the biogeography of octopodids from the region. Recent taxonomic work on the southeastern Australian fauna has facilitated a biogeographic study (see Stranks, 1988a). The present paper reports on the distribution of *Octopus* in this region related to biological and oceanographic factors.

METHODOLOGY

This study was based on a review of octopus specimens from southern Australian and New Zealand localities, held in collections in Australian museums: The Australian Museum (Sydney) (AM); Museum of Victoria (Melbourne) (NMV); Queen Victoria Museum and Art Gallery (Launceston) (QVM); South Australian Museum (Adelaide) (SAM); and Tasmanian Museum and Art Gallery (Hobart) (TMH). Additional material was examined from collections in New Zealand institutions: Canterbury Museum (Christchurch) (CMNZ); Museum of New Zealand (Wellington) (MONZ); and the Otago Museum (Dunedin) (OMNZ). Other abbreviations used are: CL, capsule length of mature ovarian or spawned egg; ML, mantle length; and TL, total length.

RESULTS

Seven valid species of octopuses have been recorded from southeastern Australian waters (Table 1).

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Octopus berrima Stranks and Norman, 1993, is one of the most common *Octopus* species in southeastern Australia, occurring from the central Great Australian Bight (about 132°E) to Twofold Bay, New South Wales (about 37°S), including Bass Strait and Tasmania (Stranks and Norman, 1993). This species produces large eggs (10-14 mm CL) that are attached singly to the substratum (see vouchers NMV F52511 and F77664), and large hatchlings (4-5 mm ML) (see voucher NMV F77665). Mature females of *O. berrima* were recorded by Tait (1980) [as *O. australis* Hoyle, 1885] brooding clutches of 52-129 eggs.

Octopus bunurong Stranks, 1990, is distributed in southeastern Australia from the central Great Australian Bight (about 132°E) to southern New South Wales (about 37°S), including Bass Strait and northern Tasmania (Stranks, 1988a; 1990). Females have medium-sized mature ovarian eggs of 8-10 mm CL (see voucher NMV F53221). Method of egg attachment, clutch size, and hatchling size are unknown.

Octopus kaurna Stranks, 1990, is recorded in southeastern Australia from the central Great Australian Bight (about 132°E) to southern New South Wales (about 37°S), including Bass Strait and northern Tasmania (Stranks, 1988a; 1990). Females have mature ovarian eggs of 9-11 mm CL (see voucher NMV F1628). Method of egg attachment, clutch size, and hatchling size are unknown.

Octopus maorum Hutton, 1880, is a common species widely distributed in southeastern Australia and New Zealand (Fig. 1). The species is recorded on continental-shelf and upper-slope regions in southeastern Australia, from the central Great Australian Bight (about 132°E) to central New South Wales (about 33°S), and also in New Zealand from the North and South islands, and Chatham, Stewart, Auckland, and Campbell islands (Dell, 1952; Stranks, 1988a). The species produces medium-sized eggs (6-7 mm CL) that are attached singly to the substratum (see vouchers SAM D17981 and OMNZ A.'44.22). Batham (1957) reported a female *O. maorum* from New Zealand with a clutch of approximately 7000 eggs, and described morphology of the paralarvae (6.7-7.6 mm TL). Additional details of planktonic hatchling morphology and size (4.3-4.5 mm ML) were given by Hochberg *et al.* (1992). Larger planktonic hatchlings of 13-17 mm ML are also recorded from off eastern Tasmania (see vouchers NMV F77607-F77610).

Octopus pallidus Hoyle, 1885, is another common species in southeastern Australia (Fig. 2), recorded from continental-shelf and upper-slope regions, ranging from the central Great Australian Bight (about 132°E) to central New South Wales (about 33°S) including Bass Strait and Tasmania (Stranks, 1988a, b). One female was observed with a spawned clutch of 270 large eggs (11-13 mm CL) that were attached singly to the substratum (see voucher NMV F52502). The species produces large hatchlings (5-6 mm ML) (see voucher NMV F31563).

Octopus superciliosus Quoy and Gaimard, 1832, is recorded in southeastern Australia from the central Great Australian Bight (about 133°E) to Twofold Bay, New South Wales (about 37°S), including Bass Strait (Stranks, 1988a). The species produces large eggs (8-11 mm CL) that are attached singly to the substratum, and large hatchlings (4-5 mm ML) (see voucher NMV F59403). Clutch size is unknown.

Octopus warringa Stranks, 1990, has a wide distribution in southeastern Australia and New Zealand; it is recorded from the central Great Australian Bight (about 125°E) to eastern Victoria (about 38°S), and also in New Zealand from the North and South islands, and Stewart Island (Dell, 1952; Stranks, 1988a; 1990). The species produces small eggs (2-3 mm CL) that are attached in festoons to the substratum (see vouchers NMV F53216 and AM C92051). Brough (1965) reported on a female *O. warringa*

Table 1. Mature female size, egg size and number, hatchling type, and general distribution of *Octopus* species in southeastern Australian waters (*, measured from mature ovarian eggs; **, estimated from number of ovarian eggs).

Species	Female size at maturity (mm ML)	Egg size (mm CL)	Egg number	Hatchling size (mm ML)	Geographical distribution
		Small to Intermediate Egg Species with Planktonic Hatchlings			
Octopus maorum	90-255	6-7	7000	4-5	SE Australia-New Zealand
O. warringa	15-30	2-3	1000	2-3	SE Australia-New Zealand
		Large Egg Species with Benthic Hatchlings			
O. berrima	30-90	10-14	50-130	4-5	SE Australia
O. bunurong	45-50	8-10*	50-100*	?	SE Australia
O. kaurna	40-85	9-11*	50-100**	?	SE Australia
O. pallidus	50-130	11-13	270	5-6	SE Australia
O. superciliosus	10-30	8-11	50-100**	4-5	SE Australia

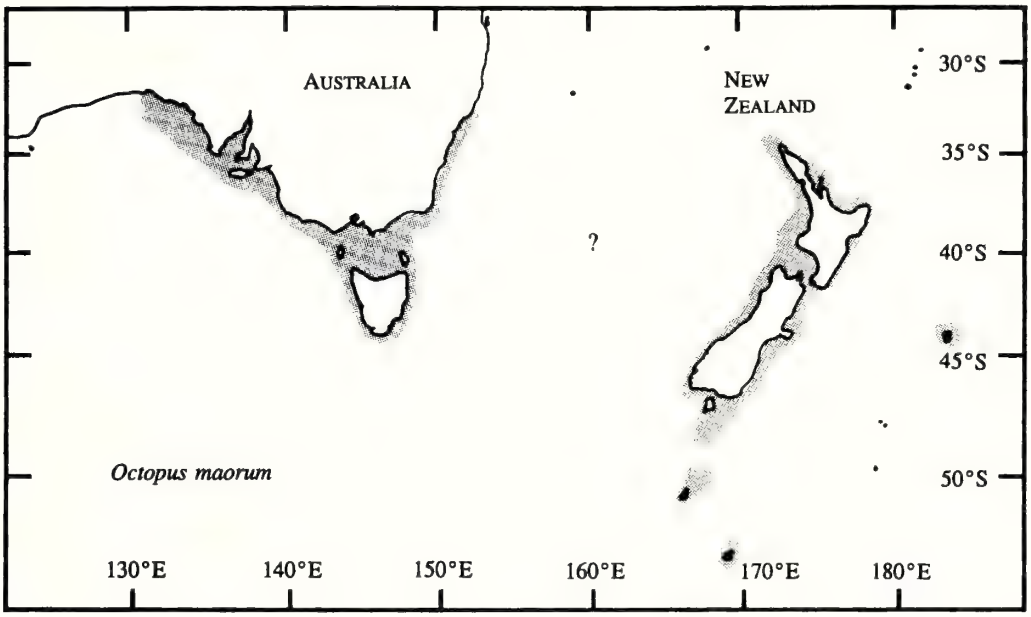
AUSTRALIA
NEW ZEALAND
?
Octopus maorum
30°S
35°S
40°S
45°S
50°S
130°E
140°E
150°E
160°E
170°E
180°E

Fig. 1. Generalized geographical distribution of small- to medium-egg sized species around the southeastern coast of Australia, and around New Zealand and adjacent islands (based on the distribution of *Octopus maorum*).

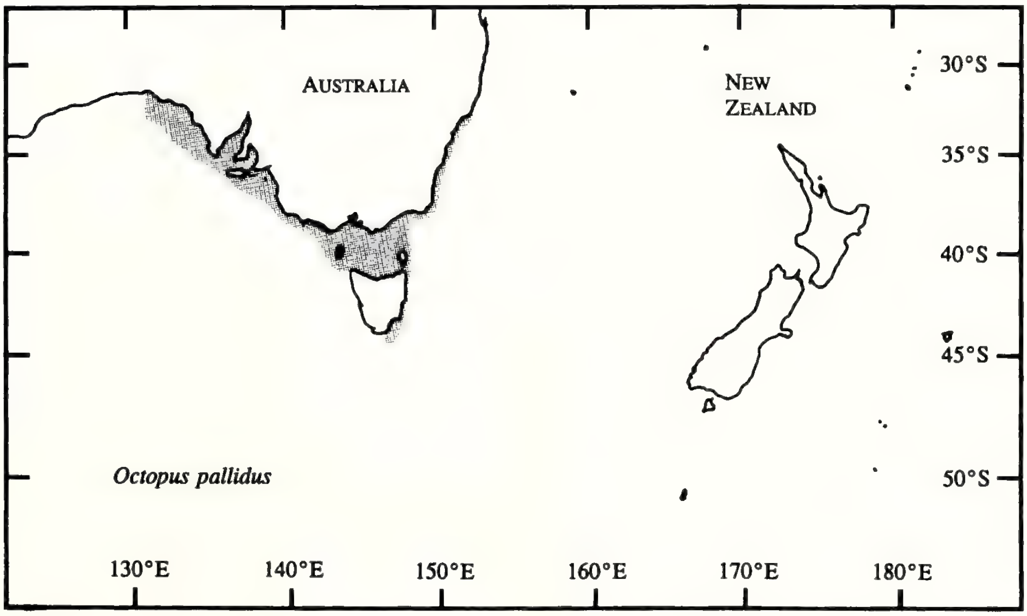
AUSTRALIA
NEW ZEALAND
Octopus pallidus
30°S
35°S
40°S
45°S
50°S
130°E
140°E
150°E
160°E
170°E
180°E

Fig. 2. Generalized geographical distribution of large egg sized species around the southeastern coast of Australia (based on the distribution of *Octopus pallidus*).

[as *Robsonella australis* (Hoyle, 1885)] brooding a clutch of about 1000 eggs, and on the morphology of the resulting hatchlings (3.6-4.0 mm TL). Further details of planktonic hatchling morphology and size (2.2-2.3 mm ML) were given by Hochberg *et al.* (1992).

DISCUSSION

The seven species of *Octopus* recorded in the present study have a localized distribution in the inshore temperate waters of southeastern Australia, and additionally with two cases, in New Zealand. The generalized distributional pattern of this species group on the Australian continent is from the Great Australian Bight to the southern or central New South Wales coast, including Bass Strait and the Tasmanian coastline. Those species also occurring in New Zealand are generally distributed around the North and South islands, and several of the smaller offshore islands. The author has not identified any of the seven *Octopus* species in areas outside these geographic limits, and on the basis of studies to date, all the species are considered endemic to the southeastern Australian and New Zealand region. This endemism at the species level corresponds well with the very high level of endemism (probably over 95%) of the southern Australian Mollusca in general (Wilson and Allen, 1987).

Adult inshore octopuses of southeastern Australia and New Zealand have a rather restricted distribution over the continental shelf and upper continental slope, from the intertidal zone to water depths of about 500 m. Adult animals can actively swim for short periods, but do not appear to migrate over extended ranges along the continental shelf, nor venture deep down the continental slope. Deep-sea trawling of mid- to lower continental slope benthos has failed to capture any of the mentioned species. Deep-sea regions could act as physical barriers to migration of these species. In this region, the Indian, Southern, and Pacific Oceans, as well as the Tasman Sea, can be considered as such oceanic barriers. Conversely, shallower stretches of water such as Bass Strait (between the Australian mainland and Tasmania) with water depths of less than 200 m do not appear to present any barrier to migration.

Apart from bathymetric isolation, other factors can influence the distribution of *Octopus* species along the continental shelf at the peripheries of their ranges. Several of the southeastern Australian species are restricted to waters east of the head of the Great Australian Bight (approximately 132°E). A local geographic feature that might present a barrier to migration of some species further west of this longitude is the cliffed Nullarbor coastline. From Eucla, Western Australia (129°E), to the head of the Great Australian Bight (132°E) is a long stretch of coast with very steep cliff frontage, extensive wave-cut platforms, and strong to extreme wave actions (Womersley and Edmonds, 1958). Whether *Octopus* species can survive in such a harsh environment is uncertain at present. Locality records for this area of the range are unfortunately sparse, so this distributional limit could to some extent be an artifact due to lack of sampling. Geographical features that might limit migration at the periphery of species' distributional ranges in eastern Australia are not obvious. Many of the *Octopus* species of southeastern Australia have ranges of distribution that extend along the eastern Australian coast as far northwards as southern or central New South Wales (32-37°S), but such distributions could be the result of hydrological rather than coastal topographical factors. This could also be reflected in the distributions of *Octopus* species around the coasts of New Zealand.

An important factor affecting the distribution of *Octopus* species in southeastern Australia and New Zealand appears to be water temperature. This temperate region has characteristic temperature profiles, described by Womersley and Edmonds (1958) and Knox (1975), that are distinct from those of neighboring areas. Distributional patterns of *Octopus* of southeastern Australia and New Zealand could be correlated with temperature regimes over these regions. Species in these temperate waters appear to tolerate temperature ranges from 10-17°C during the winter period, and 13-23°C through the summer season.

Hydrology of the temperate waters of southeastern Australia affects the distribution of inshore *Octopus* species, particularly where there is a meeting of cold and warm currents, marked by a sharp change in water temperature over a relatively short distance. The generalized temperate distribution of inshore *Octopus* species is confined in southeastern Australia, from about the center of the Great Australian Bight (130-134°E) to central or southern New South Wales (32-37°S), including Bass Strait and Tasmania. These distributional boundaries along the continental shelf in southeastern Australia could be correlated with effects of the Leeuwin Current in the west, and the East Australian Current to the east (Fig. 3). In summary, the Leeuwin Current carries warmer subtropical water around the southwestern coast of Western Australia to at least as far as the head of the Great Australian Bight (130°E) (Rochford, 1986), and can transport warm-water tropical fauna into the Bight (Maxwell and Cresswell, 1981). The East Australian Current carries warmer subtropical waters along the coast of eastern Australia, and can transport warm tropical fauna at least as far as central New South Wales (32-34°S) (Ekman, 1953).

Seawater salinity can be eliminated as a factor affecting distribution of *Octopus* species in southeastern Australia and New Zealand, as the surface salinity profiles are relatively uniform throughout this temperate region

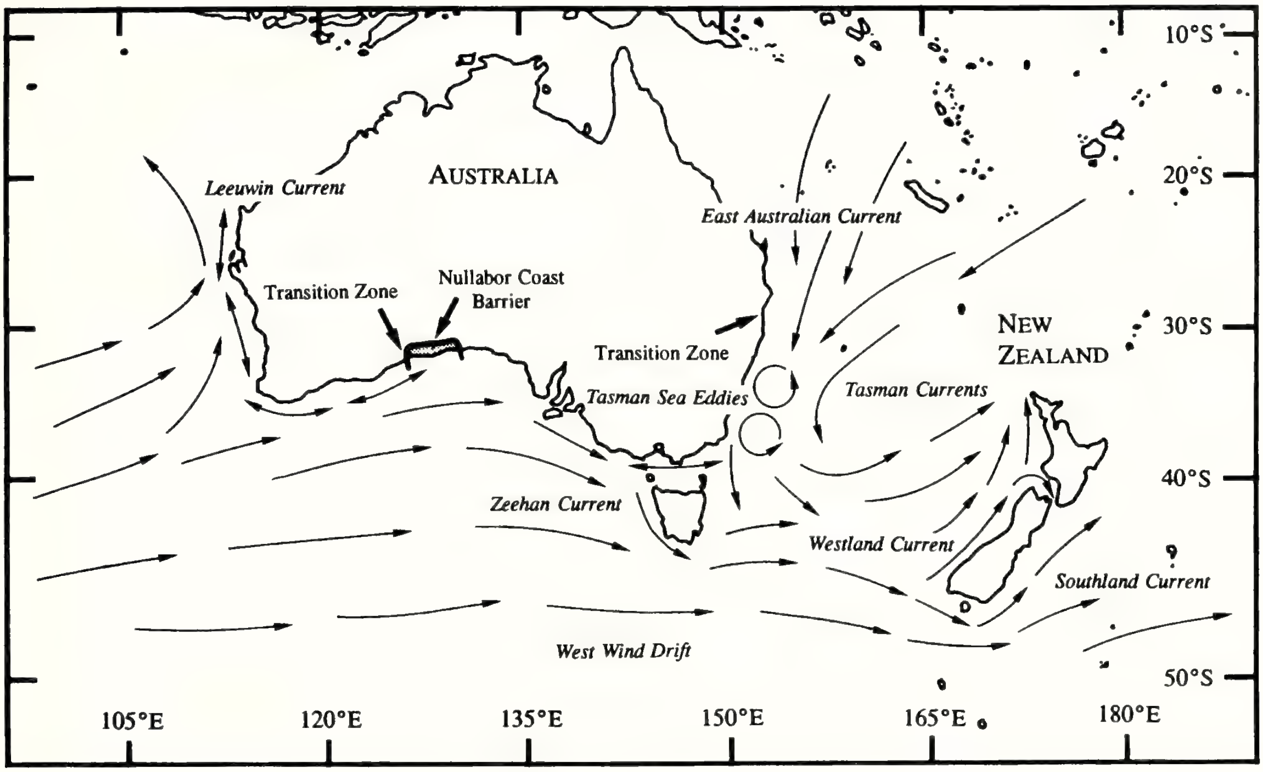

Fig. 3. Schematic chart of principal sea-surface currents influencing distribution of inshore benthic octopuses in the southeastern Australian and New Zealand region.

(Knox, 1975; Bunt, 1987).

Two alternate patterns of geographical distribution and reproductive behavior were noted. Two of the seven *Octopus* species have an extended distribution in southeastern Australian and New Zealand waters. The two taxa involved, *O. maorum* and *O. warringa*, are coincidentally the only species that produce small to intermediate sized eggs (2-7 mm CL) in very high numbers (*e. g.* 1000-7000), and whose hatchlings are relatively small and undergo a planktonic phase as paralarvae before settling out to the benthos (*i. e.* indirect development). The remaining five *Octopus* species are limited in their distribution to southeastern Australia. They produce large eggs (> 8 mm CL) in fewer numbers (*e. g.* 50-300), and the resulting hatchlings are relatively large and immediately adopt a benthic existence (*i. e.* direct development). Other mollusks that have shared distributions in Australia and New Zealand, and possess planktonic juvenile stages, have been reported by Wilson and Allen (1987).

Only the two *Octopus* species with planktonic juvenile stages occur in both Australia and New Zealand, suggesting that the distributional pattern in the region is the result of recent or on-going migration across the Tasman Sea, and not the result of previous land-mass connections between Australia and New Zealand. If the latter was the case, some of the species with non-planktonic stages might have been expected to occur in New Zealand as well as Australia.

Octopus species with benthic young do not have an alternate means for dispersal of individuals, and must rely on the relatively limited migration of adults. In other species with planktonic young, there is the potential for long-distance dispersal of juveniles across oceanic barriers. The Tasman Sea, with its submarine Tasman Trough at least 4000 m deep (Keast, 1959), appears to present a barrier to migration from southeastern Australia of *Octopus* species that have benthic juveniles and adults. However, species with benthic adults but planktonic paralarvae might have the potential for trans-Tasman migration. The distributional patterns of some *Octopus* species in southeastern Australia and New Zealand can thus be explained.

Pielou (1979) summarized requirements for successful transoceanic dispersal, noting the dependence on abundant offspring produced by the shore-dwelling adult, the probability of the juvenile being carried from coastal waters into open ocean circulation, the chances of the juvenile surviving the duration of the journey, and on chances of shifting back from oceanic to coastal waters after reaching the other shore. These factors will be examined with respect to predictions for trans-Tasman dispersal of planktonic juvenile *Octopus.*

Octopus maorum and *O. warringa* are species whose benthic adults live in inshore waters, and whose mature females are highly fecund, producing very large numbers of small or medium sized eggs, presumably to maximize chances of at least some planktonic juveniles surviving to adulthood. A mechanism for dispersal could involve hatched planktonic young being transported via the inshore coastal currents of southeastern Australia, into larger scale offshore movements of the Southern Ocean and Tasman Sea. The Tasman Currents, derived in part from the East Australian Current and also indirectly from West Wind Drift currents, appear to be the major agents for Tasman transportation. Movement of planktonic individuals in the Tasman Sea could be facilitated by strong currents and eddies. Prevailing easterly currents across the Tasman suggest that the movement and dispersal of individuals is unidirectional from Australia to New Zealand; there is no evidence for marked movement in the opposite direction. Individuals trapped in offshore Tasman Sea eddies can experience east to west transport over reduced distances for short periods, but there is no evidence that this movement would be significant enough to transport paralarvae back to shore.

At present, the duration that juveniles of *Octopus maorum* and *O. warringa* remain in the plankton before settling out to benthic existence is unknown. An indication of planktonic life is given by studies on other representatives of the genus: Yamashita (1974) calculated that juveniles of *O. dofleini* (Wülker, 1910) remain in the plankton for 2-3 months; Villanueva (1995) and Rees and Lumby (1954) estimated that juveniles of *O. vulgaris* Cuvier, 1797, remain planktonic for around two or three months respectively. Based on these figures, juveniles of *O. maorum* and *O. warringa* could have the potential to survive as plankton in oceanic currents for three months.

If juveniles of *Octopus maorum* and *O. warringa* are to reach New Zealand from Australia, the period that the juveniles remain planktonic must be correlated with the velocity of currents flowing from Australia to New Zealand. This could determine whether the young are able to be transported across the Tasman to the New Zealand coast in sufficient time, before their transition from plankton to benthos, or whether they perish before ever reaching the coast. Rates of current flow for the East Australian Current are estimated at 2 m/sec by Hamon *et al.* (1975), and for some Tasman Sea eddies at 1.3 m/sec by Cresswell (1983). Such movements for passively drifting planktonic material are deemed maximal, and will be reduced when animals are caught in slower flowing currents or entrapped in eddies. Distances from the coast of Australia to New Zealand range from approximately 1500 to 2200 km. By calculation, the fastest period in which an organism could be transported in plankton from Australia to New Zealand, under optimal conditions, would be about nine days. Because of variable current patterns and rates of flow, it is highly improbable that transportation occurs so rapidly. Nevertheless, given that *O. maorum* and *O. warringa* individuals might survive in the plankton for three months, it seems possible that juveniles could survive suspended in the plankton long enough to reach New Zealand from Australia. Hence, despite the isolation of adult populations of the two species in Australia and New Zealand, genetic exchange could be possible by continuous or sporadic transport of juvenile individuals across the Tasman Sea.

Elucidation of the distribution of *Octopus maorum* and *O. warringa* must await detailed studies of plankton systematics and ecology from off southeastern Australia and New Zealand. Collections are at hand to study the distribution of octopus paralarvae across the Tasman Sea, but until the author has completed further studies, some of the above comments must remain speculative.

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LITERATURE CITED

Batham, E. J. 1957. Care of eggs of *Octopus maorum. Transactions of the Royal Society of New Zealand* 84:629-638.

Brough, E. J. 1965. Egg care, eggs and larvae in the midget octopus, *Robsonella australis* (Hoyle). *Transactions of the Royal Society of New Zealand, Zoology* 6:7-19.

Bunt, J. S. 1987. The Australian marine environment. *In: Fauna of Australia. Vol. 1A. General Articles,* G. R. Dyne and D. W. Walton, eds. pp. 17-42. Australian Government Publishing Service, Canberra.

Cresswell, G. R. 1983. Physical evolution of Tasman Sea eddy J. *Australian Journal of Marine and Freshwater Research* 34:495-513.

Dell, R. K. 1952. The recent Cephalopoda of New Zealand. *Dominion Museum Bulletin* 16:1-157.

Ekman, S. 1953. *Zoogeography of the Sea.* Sidgwick and Jackson, London. 417 pp.

Hamon, B. V., J. S. Godfrey, and M. A. Greig. 1975. Relation between mean sea level, current and wind stress on the east coast of Australia. *Australian Journal of Marine and Freshwater Research* 26:389-403.

Hochberg, F. G., M. Nixon, and R. B. Toll. 1992. Order Octopoda Leach, 1818. *In: "Larval" and Juvenile Cephalopods: a Manual for their Identification*, M. J. Sweeney, C. F. E. Roper, K. M. Mangold, M. R. Clarke, and S. v. Boletzky, eds. *Smithsonian Contributions to Zoology* 513:213-280.

Keast, A. 1959. The Australian environment. *In: Biogeography and Ecology in Australia*, A. Keast, R. L. Crocher, and C. S. Christian, eds. pp. 15-35. Junk, The Hague.

Knox, G. A. 1975. The marine benthic ecology and biogeography. *In: Biogeography and Ecology in New Zealand,* G. Kuschel, ed. pp. 353-403. Junk, The Hague.

Lu, C. C. and M. C. Dunning. In press. Subclass Coleoidea. I*n: Fauna of Australia. Vol. 5. Mollusca.* Australian Government Publishing Service, Canberra.

Lu, C. C. and J. U. Phillips. 1985. An annotated checklist of the Cephalopoda from Australian waters. *Occasional Papers from the Museum of Victoria* 2:21-36.

Maxwell, J. G. H. and G. R. Cresswell. 1981. Dispersal of tropical marine fauna to the Great Australian Bight by the Leeuwin Current. *Australian Journal of Marine and Freshwater Research* 32:493-500.

Nesis, K. N. 1987. *Cephalopods of the World; Squids, Cuttlefishes, Octopuses, and Allies.* T. F. H. Publications, Neptune City, New Jersey. 351 pp.

Norman, M. D. 1992a. *Octopus cyanea* Gray, 1849 (Mollusca: Cephalopoda) in Australian waters: description, distribution and taxonomy. *Bulletin of Marine Science* 49:20-38.

Norman, M. D. 1992b. *Systematics and Biogeography of the Shallow-water Octopuses (Cephalopoda: Octopodidae) of the Great Barrier Reef, Australia.* Doctoral Thesis, University of Melbourne, Australia. 281 pp.

Norman, M. D. 1993a. Four new octopus species of the *Octopus macropus* group (Cephalopoda: Octopodidae) from the Great Barrier Reef, Australia. *Memoirs of the Museum of Victoria* 53:267-308.

Norman, M. D. 1993b. Ocellate octopuses (Cephalopoda: Octopodidae) of the Great Barrier Reef, Australia: description of two new species and redescription of *Octopus polyzenia* Gray, 1849. *Memoirs of the Museum of Victoria* 53:309-344.

Norman, M. D. 1993c. *Octopus ornatus* Gould, 1852 (Cephalopoda: Octopodidae) in Australian waters: morphology, distribution, and life history. *Proceedings of the Biological Society of Washington* 106:645-660.

O'Shea, S. J. 1990. *The Systematics of the New Zealand Octopodidae (Cephalopoda: Octopoda).* Master's Thesis, University of Auckland, New Zealand. 136 pp.

Pielou, E. C. 1979. *Biogeography.* Wiley and Sons, New York. 351 pp.

Rees, W. J. and J. R. Lumby. 1954. The abundance of octopus in the English Channel. *Journal of the Marine Biological Association of the United Kingdom* 33:515-536.

Rochford, D. J. 1986. Seasonal changes in the distribution of Leeuwin Current waters off southern Australia. *Australian Journal of Marine and Freshwater Research* 37:1-10.

Stranks, T. N. 1988a. *Systematics of the Family Octopodidae (Mollusca: Cephalopoda) of Southeastern Australia.* Master's Thesis, University of Melbourne, Australia. 114 pp.

Stranks, T. N. 1988b. Redescription of *Octopus pallidus* (Cephalopoda: Octopodidae) from southeastern Australia. *Malacologia* 29:275-287.

Stranks, T. N. 1990. Three new species of *Octopus* (Mollusca: Cephalopoda) from southeastern Australia. *Memoirs of the Museum of Victoria* 50:457-465.

Stranks, T. N. In press. The systematic and nomenclatural status of the Octopodinae described from Australia (Mollusca: Cephalopoda). *Smithsonian Contributions to Zoology.*

Stranks, T. N. and M. D. Norman. 1993. Review of the *Octopus australis* complex from Australia and New Zealand, with description of a new species (Mollusca: Cephalopoda). *Memoirs of the Museum of Victoria* 53:345-373.

Tait, R. W. 1980. *Aspects of the Ecology and Life History of* Octopus australis *Hoyle, from Northern Port Phillip Bay.* Bachelor's Thesis, Monash University, Australia. 77 pp.

Villanueva, R. 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. *Canadian Journal of Fisheries and Aquatic Science* 52:2639-2650.

Wilson, B. R. and G. R. Allen. 1987. Major components and distribution of marine fauna. *In: Fauna of Australia. Vol. 1A. General Articles,* G. R. Dyne and D. W. Walton, eds. pp. 43-68. Australian Government Publishing Service, Canberra.

Womersley, H. B. S. and S. J. Edmonds. 1958. A general account of the intertidal ecology of south Australian coasts. *Australian Journal of Marine and Freshwater Research* 9:217-260, pls. 1-12.

Yamashita, Y. 1974. Spawning and hatching of *Paroctopus dofleini dofleini* (Wülker). *Monthly Report of the Hokkaido Fisheries Experimental Station* 31:10-22.

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63rd ANNUAL MEETING
THE AMERICAN MALACOLOGICAL UNION
SANTA BARBARA, CALIFORNIA
JUNE 22-27, 1997

The 1997 meeting of the American Malacological Union will be held at the Radisson Hotel on the beach in Santa Barbara, California, from Sunday, June 22, to Friday, June 27. The meeting will be held jointly with the 30th meeting of the Western Society of Malacologists.

Two major symposia are scheduled:

(1) Deep-Sea Mollusca, convened by Jerry Harasewych [Division of Mollusks, National Museum of Natural History, Washington, DC 20560; (202) 786-2073; FAX: (202) 357-2343; mnhiv006@sivm.si.edu], an overview of the fauna of the deep sea and what it tells us about the evolution of the Mollusca and its adaptation to the deep sea.

(2) Traditional vs. Phylogenetic Systematics, convened by Gary Rosenberg [Malacology, The Academy of Natural Sciences, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103-1195; (215) 299-1033; FAX: (215) 299-1170; rosenberg@say.acnatsci.org].

There will also be a special session on the cephalopods of the North Pacific chaired by Eric Hochberg [Invertebrate Zoology, Santa Barbara Museum of Natural History, 2559 Puesta del Sol Rd., Santa Barbara, CA 93019; (805) 682-4711, ext. 318; FAX: (805) 569-3170; inverts@sbmnh.rain.org].

There will be a reception the first evening on the beach across from the hotel, a visit to a winery in the Cachuma Valley, and a banquet at the Santa Barbara Museum. Optional field trips on the 27th include a tour of fossil formations of the Santa Barbara area led by Lindsey T. Groves [Malacology, Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, CA 90007; (213) 744-3376, or -3485; FAX: (213) 746-2999; groves@usc.edu], a cruise to the Channel Islands, and an in-depth tour of the Santa Barbara Museum of Natural History.

Those traveling from the East Coast or Midwest may want to arrive on the West Coast a day earlier or to stay two days later to get the lowest possible air fares. The Radisson Hotel will be $109 per room [double occupany; i. e. $55/person]; lower-cost options for students will be available. There are a number of nearby excellent restaurants in a variety of price classes.

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IN MEMORIAM

R. Tucker Abbott

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Hillis, D. M. 1989. Genetic consequences of partial self fertilization on population of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* 7(1):7-12.

Seed, R. 1980. Shell growth and form in the Bivalvia. *In: Skeletal Growth of Aquatic Organisms,* D. C. Rhoads and R. A. Lutz, eds. pp. 23-67. Plenum Press, New York.

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Review of the family Phyllidiidae in the Atlantic Ocean (Nudibranchia, Doridoidea)

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Abstract: The species of Phyllidiidae from the Atlantic Ocean (Mediterranean Sea excluded) are redescribed in this paper. In light of the study of type material and newly collected specimens, we concluded that *Phyllidiopsis gynenopla* Bouchet, 1977, is a junior synonym of *P. berghi* Vayssière, 1902. Also, anatomical studies supported the placement of *P. papilligera* Bergh, 1890, in the genus *Ceratophyllidia* Eliot, 1903, and *Phyllidiopsis molaensis* Meyer, 1977, in the genus *Phyllidiella* Bergh, 1869. Two new bathyal species, *Reticulidia gofasi* n. sp. and *Phyllidiopsis boucheti* n. sp., are described. The former is the first record of this genus in the Atlantic Ocean.

Key words: Nudibranchia, Phyllidiidae, taxonomy, new species, Atlantic Ocean

A review of the literature shows that few nominal species of the family Phyllidiidae, all lacking bright colors, have been described in the Atlantic Ocean (Mediterranean Sea excluded). This contrasts with the richly and brightly colored phyllidiid fauna of the Indo-Pacific recently reviewed by Brunckhorst (1993).

The first report of this family in Atlantic waters was the description of *Phyllidiopsis papilligera* by Bergh (1890), based on a single specimen collected at 182 m depth from the Gulf of Mexico, during the *Blake* Expedition. *P. papilligera* was later redescribed by Marcus and Marcus (1962, 1967) and Thompson (1980). Several years later, Vayssière (1902b) described *Phyllidiopsis berghi*, a species of uniform white color collected at 1480 m depth from the Bay of Biscay, by the *Talisman* Expedition. In 1977, two new species of *Phyllidiopsis* were reported from the Atlantic Ocean. One of them, *P. gynenopla* Bouchet, 1977, was described on the basis of a single specimen collected at 525-600 m, near the Azores by the *Biaçores* Expedition. In the same paper, Bouchet (1977) redescribed *P. berghi.* The other species, *P. molaensis*, was described by Meyer (1977) from shallow waters of the Caribbean coast of Panama.

In the present paper, we review all the species reported in the Atlantic Ocean, and describe two new species. The Mediterranean species, *Phyllidia flava* Aradas, 1847, and *Fryeria bayi* Bouchet, 1983, have not been included because they were recently reviewed by Brunckhorst (1993).

MATERIAL AND METHODS

Most of the specimens studied were collected during several scientific expeditions (*Z-Thalassa, Biaçores, Seamount I,* and *Seamount II*) organized by the Muséum National d'Histoire Naturelle, Paris, France (MNHN) with the vessels of the French agency for oceanographic research (IFREMER, Institut Français de Recherche pour l'Exploitation de la Mer). Also, type material was examined from the collections of MNHN and the National Museum of Natural History, Washington D. C. (USNM).

Features of living specimens were recorded from original notes and drawings of collectors. Several specimens have been dissected and particularly interesting soft parts have been critical point dried for scanning electron micrography (SEM).

SPECIES DESCRIPTIONS

Genus *Phyllidiella* Bergh, 1869
Type species *Phyllidia pustulosa* Cuvier, 1804, by subsequent designation (Brunckhorst, 1993).

Phyllidiella molaensis (Meyer, 1977)
(Figs. 1A, 2)

Phyllidiopsis molaensis Meyer, 1977: 305-306, fig. 4.

Material Examined
USNM 760616, HOLOTYPE (by original designation),

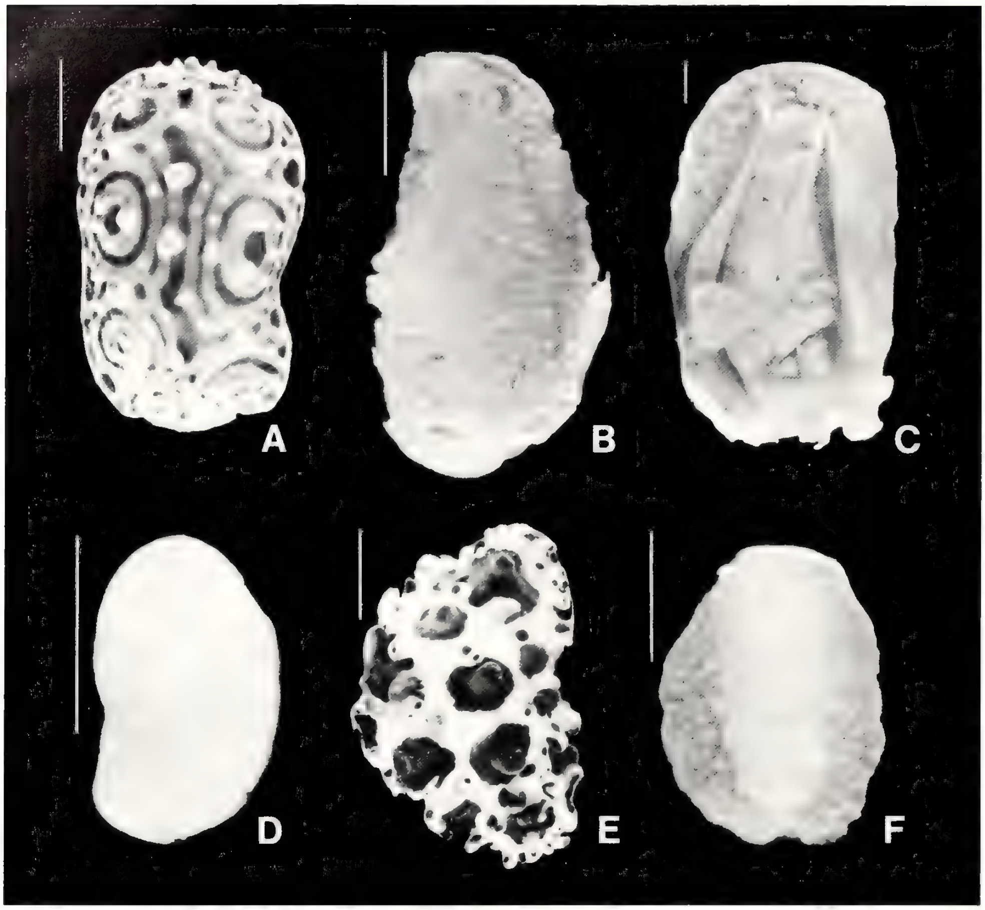

Fig. 1. Dorsal views of preserved type specimens (scale bars = 5 mm). **A.** *Phyllidiella molaensis*, holotype (USNM 760616). **B.** *Phyllidiopsis berghi*, holotype (MNHN). **C.** *P. berghi*, holotype of *Phyllidiopsis gynenopla* (MNHN). **D.** *Phyllidiopsis boucheti*, holotype (MNHN). **E.** *Ceratophyllidia papilligera*, neotype (USNM 856418). **F.** *Reticulidia gofasi*, holotype (MNHN).

Galeta Point, Panama, 15 m depth, 25 May 1971, 20 mm preserved length, coll. Meyer.

USNM 760617, PARATYPE, Portobelo, Panama, 9 m depth, 10 September 1971, 1 spm, 19 mm preserved length, coll. Meyer.

External Morphology

The color in life of this species was described by Meyer (1977) as '...color black and white consisting of three sets of concentric circles along the sides, two more anteriorly and one set posteriorly, each set composed of two concentric white rings each of which has a grey ring running through its middle and separated from one another by a black ring; white lines randomly traversing areas not covered by the rings; rhinophores black with white tips...' In preserved specimens only brown shadows remain of the original black color (Fig. 1A). The dorsum bears simple, conical tubercles, decreasing in size toward the notal margin. The mantle margin is as wide as the notum. The anus opens dorsally. Rhinotubercles (see Brunckhorst, 1993) are absent.

Ventrally, the oral tentacles are separated. The anterior and posterior ends of the foot are notched (Figs. 2B-C). The branchial leaves are very thin and elongated, with alternating large and small ones (Fig. 2D).

Anatomy

The moderately long oral tube dilates into a very folded pharyngeal bulb (Fig. 2A). Two anterior retractor muscles insert at the point where the pharyngeal bulb and oral tube connect; another pair of muscles inserts in both sides of the pharyngeal bulb. The esophagus is long, with no muscular or glandular portions.

Geographical Range

This species has only been recorded from the Caribbean coast of Panama.

Discussion

Anatomical features of this species resemble those

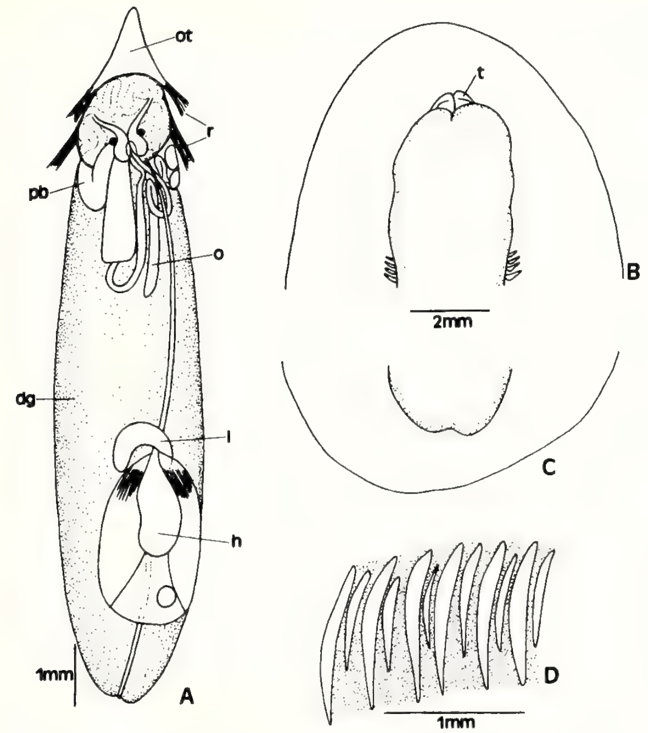

Fig. 2. *Phyllidiella molaensis*, paratype (USNM 760617). **A.** Dorsal view of the anatomy. **B.** Ventral view of the anterior edge of the foot showing the oral tentacles. **C.** Ventral view of the posterior edge of the foot. **D.** Detail of the branchial leaves. dg, digestive gland; h, heart; i, intestine; o, esophagus; ot, oral tube; pb, pharyngeal bulb; r, retractor muscles; t, oral tentacles.

described by Brunckhorst (1993) for the genus *Phyllidiella*, such as the structure of the digestive system with a moderately long oral tube which connects with a folded pharyngeal bulb. Externally, this species has separate oral tentacles, simple tubercles on the dorsum, black rhinophores, and no rhinotubercles, a combination of characters only present in the species of this genus (Brunckhorst, 1993).

Genus *Phyllidiopsis* Bergh, 1875

Type species *Phyllidiopsis cardinalis* Bergh, 1875, by monotypy.

Phyllidiopsis berghi Vayssière, 1902
(Figs. 1B-C, 3, 4A-B, 4D)

Phyllidiopsis berghi Vayssière, 1902a: 1-2 (*nomen nudum*); 1902b: 237-242, pls. 9-10; Bouchet, 1977: 48-49, figs. 16-17. *Phyllidiopsis gynenopla* Bouchet, 1977: 50-53, figs. 18-19, syn. nov.

Material Examined

MNHN, HOLOTYPE of *P. berghi* (by monotypy), *Talisman* sta. 141 (45°59.00' N, 04°09.46' W), 1480 m depth, 30 August 1883, 16 mm preserved length, dissected.

MNHN, HOLOTYPE of *P. gynenopla* (by original designation), *Jean Charcot-Biaçores* sta. 159 (37°26.00' N, 25°51.00' W), 525-600 m depth, 31 October 1971, 48 mm preserved length, dissected.

MNHN, *Jean Charcot-Biaçores* sta. 59 (38°22.05' N, 28°48.05' W), 560-580 m depth, 14 October 1971, 2 spms, 7 and 9 mm preserved length.

MNHN, *Z-Thalassa* sta. 435 (48°39.07' N, 09°53.02' W), 1050 m depth, 26 October 1973, 1 spm, 14 mm preserved length, dissected.

MNHN, *Seamount II* sta. DW128, Gran Canaria (28°08.30'

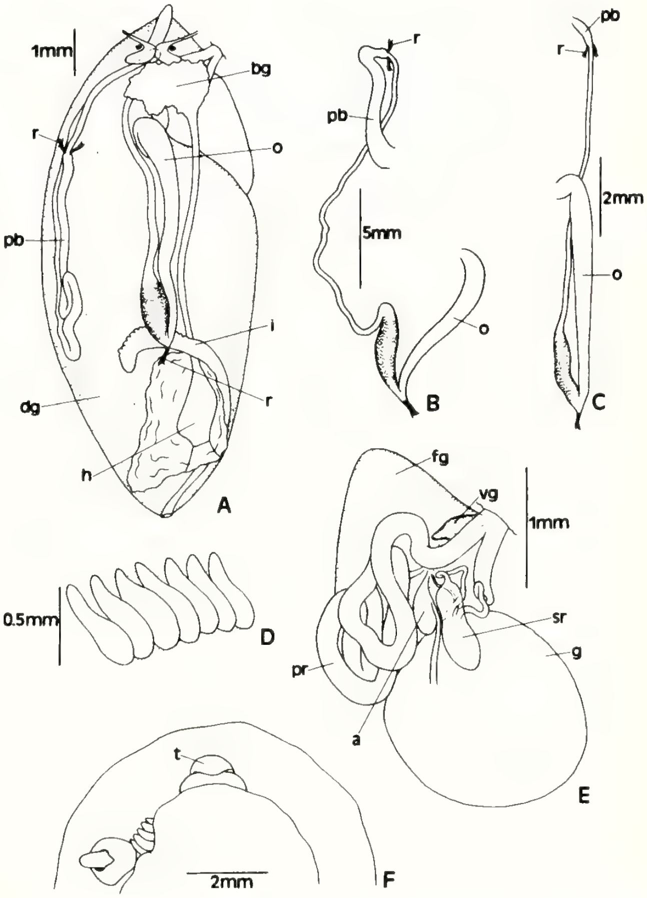

Fig. 3. *Phyllidiopsis berghi.* **A.** Dorsal view of the anatomy of the specimen from Gran Canaria (MNHN). **B.** Detail of the anterior digestive tract dissected from the holotype of *Phyllidiopsis gynenopla* (MNHN). **C.** Detail of the anterior digestive tract dissected from the holotype of *P. berghi* (MNHN). **D.** Detail of the branchial leaves. **E.** Genital system. **F.** Ventral view of preserved holotype of *P. gynenopla* showing the oral tentacles. a, ampulla; bg, blood gland; dg, digestive gland; fg, female gland; g, gametolytic gland; h, heart; i, intestine; o, esophagus; pb, pharyngeal bulb; pr, prostate; r, retractor muscles; sr, seminal receptacle; t, oral tentacles; vg, vestibular gland.

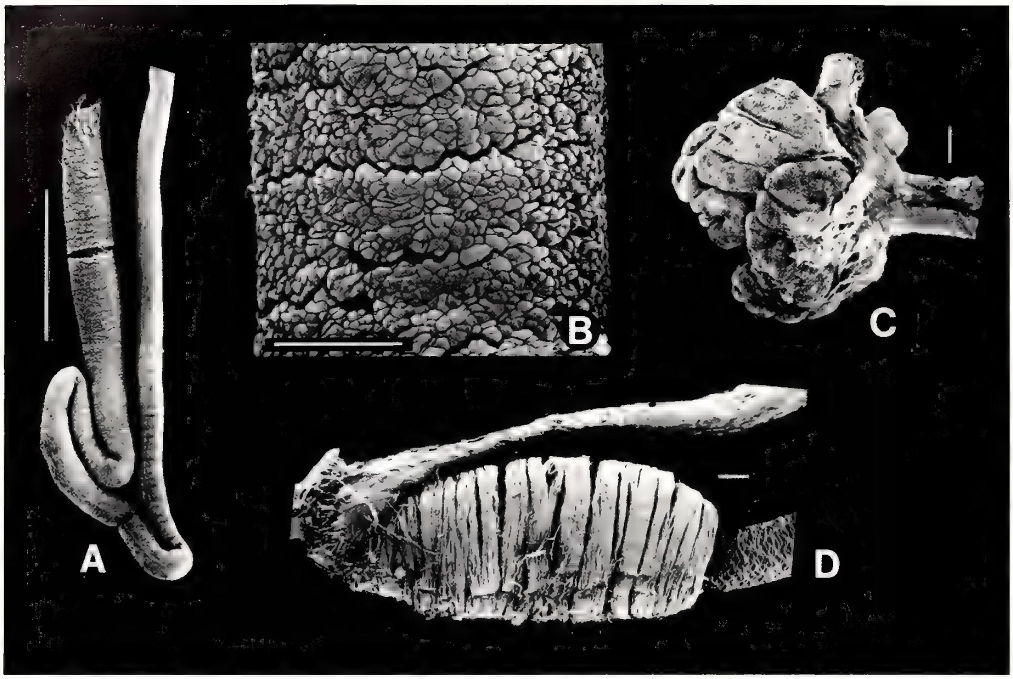

Fig. 4. Scanning electron micrographs (SEM) using critical point drying technique. **A.** Pharyngeal bulb of *Phyllidiopsis berghi.* **B.** Detail of the external glands of the pharyngeal bulb of *P. berghi.* **C.** Pharyngeal bulb of *Reticulidia gofasi.* **D.** Muscular esophageal portion of digestive tract of *P. berghi.* Scale bars = 1 mm (A), 100 µm (B, D), 200 µm (C).

N, 15°52.00' W), 470 m depth, 06 January 1993, 1 spm, 13 mm preserved length, dissected.

MNHN, *Seamount II* sta. DW188, Hyères Bank (31°30.00' N, 28°59.50' W), 310 m depth, 17 January 1993, 1 spm, 7 mm preserved length.

MNHN, *Seamount II* sta. DW200, Hyères Bank (31°19.10' N, 28°36.00' W), 1060 m depth, 17 January 1993, 1 spm, 8 mm preserved length.

MNHN, *Seamount II* sta. DW241, Plato Bank (33°11.90' N, 28°50.30' W), 695 m depth, 31 January 1993, 2 spms, 6 and 9 mm preserved length.

External Morphology

The general body color of live animals is pale cream; in larger specimens black pigment over several tubercles has been described (Bouchet, 1977). The dorsum bears numerous rounded tubercles (Figs. 1B-C). Several larger tubercles are distributed among the others in adult specimens. The border of the mantle is narrow, nearly 1/10 of the notum width. The rhinophores are white. The anus opens dorsally in all specimens observed. Rhinotubercles are absent.

Ventrally, the edge of the mantle shows spicules in reticular disposition. The anterior edge of the foot is not notched and the oral tentacles are spherical and fused (Fig. 3F). The branchial leaves are wide, triangular, and equal in size (Fig. 3D). They are clustered very closely together.

Anatomy

The pharyngeal bulb is very long (Figs. 3A, 4A) and covered by numerous minute glands (Fig. 4B). Two anterior retractor muscles insert at the point where the pharyngeal bulb and the esophagus connect. The esophagus is also very long, with a muscular portion near the end (Fig. 4D) where the posterior retractor muscles are attached. The intestine presents several small tubercles on its anterior portion.

The genital system (Fig. 3E) has a vestibular gland. The ampulla is smaller than the seminal receptacle in all specimens examined. From the gametolytic gland emerge three ducts, one of them connecting with the seminal receptacle, another with the vagina, and the thinnest of them with the female gland. The prostatic portion of the deferent duct is long and folded.

Geographical Range

This species, occurring in deep water, is only known in the North Atlantic (Fig. 5). It has been collected on the continental slope of France, in the Azores (slope of São Jorge), on the seamounts of the Meteor Group (Hyères Bank, Plato Bank), and in the Canary Islands (slope of Gran Canaria).

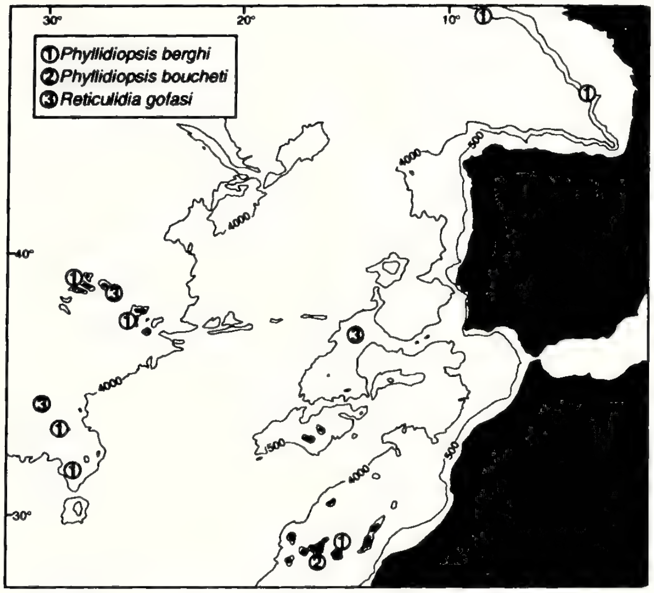

Fig. 5. Distribution map of the eastern Atlantic phyllidiid species.

Discussion

Vayssière (1902a) introduced the name *Phyllidiopsis berghi* but did not give a description (*nomen nudum*). Several months later, Vayssière (1902b) gave a exhaustive description of this species with anatomical details.

Phyllidiopsis gynenopla was described as different from *P. berghi* by Bouchet (1977), on the basis of the distinctive external color pattern and few anatomical details. Observation of the digestive tract of the holotypes of both nominal species showed no significant differences (Figs. 3B-C). Also, the external morphology is identical among the specimens observed. The difference in the external color can be explained by the difference in size (more than 30 mm) between the holotype of *P. gynenopla* and other material examined here assigned to *P. berghi*.

Another species with color pattern similar to *Phyllidiopsis berghi* is *P. blanca* Gosliner and Behrens, 1988, from the Pacific coast of North America. However, that species is distinguished by the disposition of the branchial leaves, all equal in size in *P. berghi*, and with alternation of large and small in *P. blanca* (see Gosliner and Behrens, 1988). Also, the disposition of the dorsal tubercles is different in the two species. In *P. berghi*, they are very close together, and there are two different sizes of tubercles; in *P. blanca* they are scattered and all of them are the same size. Anatomical differences between these species are found in the different disposition of the ducts arranged from the gametolytic gland (three in *P. berghi* and one in *P. blanca*).

Phyllidiopsis boucheti new species

(Figs. 1D, 6)

Material Examined

MNHN, HOLOTYPE, Punta de la Rasca, Tenerife, Canary Islands, 400 m depth, 12 May 1988, 8 mm preserved length, leg. J. J. Bacallado; MNHN, PARATYPE, same locality, 1 spm, 38 mm preserved length, dissected, leg. J. J. Bacallado.

External Morphology

Both specimens were fixed, so that no external features of the living animals were available. The general body color is white in the preserved specimens (Fig. 1D). The dorsum is entirely covered by numerous, minute, simply rounded tubercles. No gradation in size occurs in the tubercles near the notal margin. The rhinophores are white. Rhinotubercles are absent. The mantle margin is as wide as 1/3 of the notum. The anus opens dorsally in all specimens observed.

Ventrally, the branchial leaves are triangular (Fig. 6C). The oral tentacles are long and fused together (Fig. 6B). The mantle margin has spicules in reticular disposition. The anterior edge of the foot is not notched.

Anatomy

The esophagus is very thin and long (Fig. 6A). It has a muscular area in the middle of its length, just behind the intestine. The pharynx is elongate, but approximately half the length of the esophagus.

The reproductive system (Fig. 6A) is bordered by the digestive gland. The smooth prostatic portion of the deferent duct is twice as long as the ampulla, and leads into the short and narrow deferent duct.

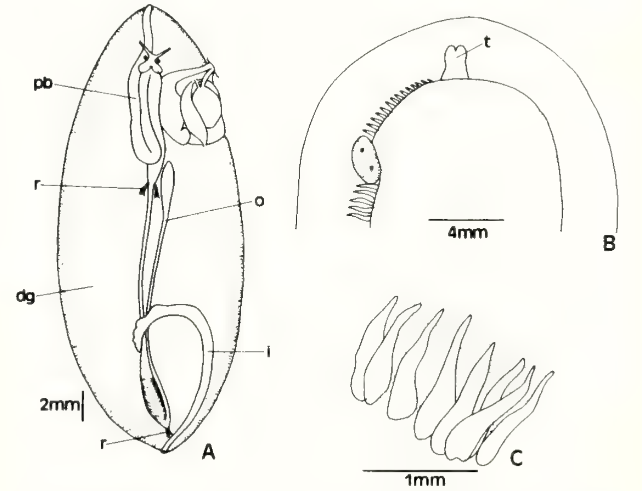

Fig. 6. *Phyllidiopsis boucheti,* paratype (MNHN). **A.** Dorsal view of the anatomy. **B.** Ventral view of preserved specimen showing the oral tentacles. **C.** Detail of the branchial leaves. dg, digestive gland; i, intestine; o, esophagus; pb, pharyngeal bulb; r, retractor muscles; t, oral tentacles.

Geographical Range

This species, occurring in deep water, is only known from the southern slope of Tenerife, Canary Islands (Fig. 5).

Discussion

Phyllidiopsis boucheti is clearly different from *P. berghi*. The species differ in the size, shape, and distribution of the dorsal tubercles; they are very minute and even in size in *P. boucheti,* and larger, and of two different sizes in *P. berghi*. Also, ventrally, the shape and disposition of the branchial leaves are different in the two species.

The differences between *Phyllidiopsis boucheti* and *P. blanca* are the shape and disposition of the dorsal tubercles, very minute and close together in the former and large and scattered in the latter. Also, differences in the shape and disposition of the branchial leaves support separation of the two species.

Brunckhorst (1990b, 1993) has suggested that the nominal species *Phyllidiopsis berghi* and *P. gynenopla*, from the Atlantic Ocean, and *P. blanca*, from the Pacific coast of Northern America, should be included in a new genus, because the published descriptions make no mention of anterior retractor muscles and muscular segments in the esophagus of these species. Detailed anatomical studies of our material shows that Atlantic *Phyllidiopsis* do have anterior retractor muscles and also that there is a muscular portion in the esophagus as in the Indo-Pacific species. New anatomical studies of *P. blanca* will probably also show the presence of these features. Although Atlantic and Indo-Pacific *Phyllidiopsis* show differences, such as the longer pharyngeal bulb in the Atlantic species, we feel that this is not enough to consider the separation of the Atlantic and Indo-Pacific *Phyllidiopsis* as two different genera.

Etymology

The name *Phyllidiopsis boucheti* is in honor of our friend and colleague, Dr. Philippe Bouchet (MNHN), who has made important contributions to the knowledge of mollusks.

Genus *Ceratophyllidia* Eliot, 1903
Type species *Ceratophyllidia africana* Eliot, 1903, by monotypy.

Ceratophyllidia papilligera (Bergh, 1890)

(Figs. 1E, 7)

Phyllidiopsis papilligera Bergh, 1890: 176-178, pls. 2, 7-14; Marcus and Marcus, 1962: 475-479, figs. 20-24; 1967: 99, fig. 123; Thompson, 1980: 93, fig. 12.

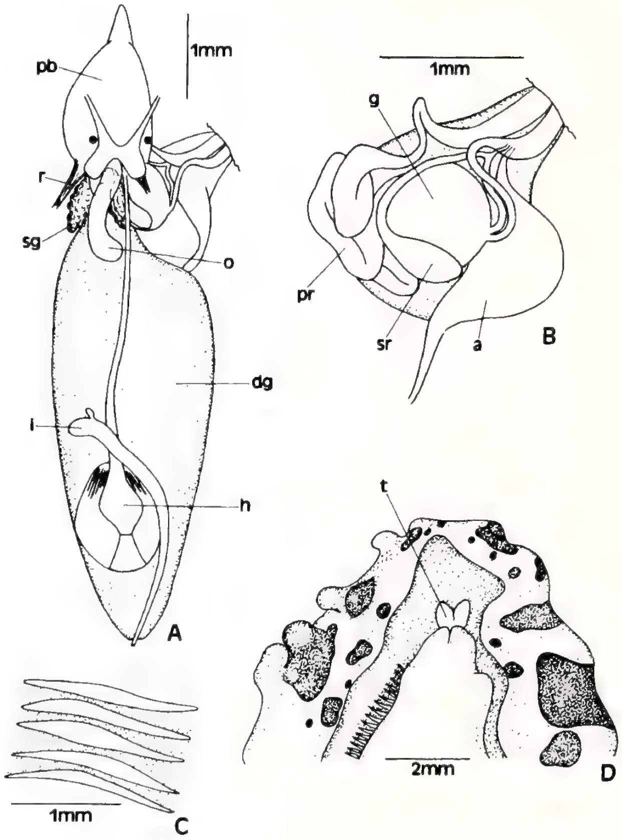

Fig. 7. *Ceratophyllidia papilligera*. **A.** Dorsal view of the anatomy. **B.** Genital system. **C.** Detail of the branchial leaves. **D.** Ventral view of preserved neotype (USNM 856418) showing the oral tentacles. a, ampulla; dg, digestive gland; g, gametolytic gland; h, heart; i, intestine; o, esophagus; pb, pharyngeal bulb; pr, prostate; r, retractor muscles; sg, salivary glands; sr, seminal receptacle; t, oral tentacles.

Material Examined

USNM 856418, NEOTYPE, *Sofla* Expedition sta. 11, (26°16.43' N, 83°46.49' W), 77 m depth, 30 April 1981, 19 mm preserved length.

USNM 856417, *Sofla* Expedition sta. 11, (26°16.43' N, 83°46.49' W), 77 m depth, 30 April 1981, 2 spms, 10 and 12 mm preserved length.

Indian Keys, Cuba, 30 October 1993, 30 m depth, 1 spm, 26 mm long, leg. J. Espinosa.

External Morphology

The general color of the body is white, with rounded black spots of various sizes (Fig. 1E). The body is oval-shaped, with numerous stalked papillae on the dorsum. The placement of the black spots is independent of the papillae; their size is variable but usually very large. The notum is spiculose. The rhinophores are white with black lamellae; each clavus has ten lamellae. Rhinotubercles are absent.

Ventrally, the anterior border of the foot is notched (Fig. 7D). The dark tubercles of the notal margin show through the ventral side. The oral tentacles are separated. The branchial leaves are equal in size (Fig. 7C).

Anatomy

At the point where the esophagus inserts into the musculoglandular pharyngeal bulb, it connects with two conspicuous and brown-colored salivary glands (Fig. 7A). The esophagus is short, and no glandular portion has been observed.

The genital system (Fig. 7B) shows an ampulla larger than the gametolytic gland. No vestibular gland has been observed. The gametolytic gland connects with two ducts, one of them connecting with the vagina, and the other one with the seminal receptacle and the female gland.

Geographical Range

This species appears to be restricted to the Caribbean Sea and Gulf of Mexico. After its original description from the Gulf of Mexico (Bergh, 1890), *Ceratophyllidia papilligera* has been reported from Jamaica (Marcus and Marcus, 1967; Thompson, 1980) and from the Virgin Islands and Bahamas (Marcus and Marcus, 1962).

Discussion

Eliot (1903b) described the new genus *Ceratophyllidia* and included *Phyllidiopsis papilligera* as the type species. In a later paper (Eliot, 1903a), he described the new species *Ceratophyllidia africana*, including a description of the genus *Ceratophyllidia*. According to Brunckhorst (1990b, 1993), the former paper was received in June 1902 but was not published until sometime between July and November 1903. Eliot's description of *C. africana* appeared earlier (March 1903) and therefore constitutes the original description of the genus *Ceratophyllidia* with *C. africana* as the type species by monotypy. The anatomical characters observed in *C. papilligera* present all the features characteristic of the genus *Ceratophyllidia* described by Eliot (1903a, b) and Brunckhorst (1993) based on *C. africana*. The structure of the digestive system, with two conspicuous salivary glands, and the external stalked papillae of *C. papilligera* confirm its placement in the genus *Ceratophyllidia*.

Phyllidiella molaensis shows an external combination of colors similar to *Ceratophyllidia papilligera*. Nevertheless, anatomical features such as the structure of the digestive system (typical of the genus *Phyllidiella* in *P. molaensis*) and external differences such as the shape of the dorsal tubercles (conical tubercles in *P. molaensis* and stalked papillae in *C. papilligera*), the disposition of the branchial leaves (showing alternation in *P. molaensis* and equal in size in *C. papilligera*), and the pattern of color (black rings in *P. molaensis* and black spots in *C. papilligera*) easily separate the two species.

According to Thompson (1980), the type material of *Ceratophyllidia papilligera* is untraceable; however, for nomenclatural stability of this species we considered it desirable to designate a neotype. For this reason, we designated as neotype one specimen, USNM 856418, collected in the Gulf of Mexico, off Florida, close to the original record of this species.

Genus *Reticulidia* Brunckhorst, 1990
Type species *Reticulidia halgerda* Brunckhorst and Burn, 1990, by original designation.

Reticulidia gofasi new species
(Figs. 1F, 4C, 8)

Material Examined

MNHN, HOLOTYPE, *Seamount I* sta. DW61, Josephine Bank (36°40.02' N, 14°16.00' W), 200-205 m depth, 07 October 1987, 11 mm preserved length.

MNHN, PARATYPE, *Seamount II* sta. DW256, Atlantis Bank (34°06.20' N, 30°16.00' W), 340 m depth, 02 February 1993, 1 spm, 9 mm preserved length, dissected.

MNHN, PARATYPE, *Jean Charcot-Biaçores* sta. 11 (38°30.00' N, 27°14.05' W), 76-105 m depth, 08 October 1971, 1 spm, 10 mm preserved length, dissected.

MNHN, *Seamount II* sta. DW274, Atlantis Bank (34°05.10' N, 30°13.60' W), 280 m depth, 05 February 1993, 1 spm, 3 mm preserved length.

External Morphology

The general body color is pale yellow in live specimens. The dorsum is heavily spiculose, covered by conical tubercles, grading to smaller toward the notal margin (Fig. 1F). The mantle margin is as wide as the notum, and translucent. The rhinophores are pale yellow. Rhinotubercles are absent. The anus opens dorsally in all specimens observed.

Ventrally, the anterior edge of the foot is notched (Fig. 8D). The large and conical oral tentacles are separated. The branchial leaves are very thin, and triangular, showing alternation of large and small ones (Fig. 8C). The mantle margin shows radial spicules.

Anatomy

The pharyngeal bulb is embedded on its anterior portion in the transparent oral tube; its edge is surrounded by

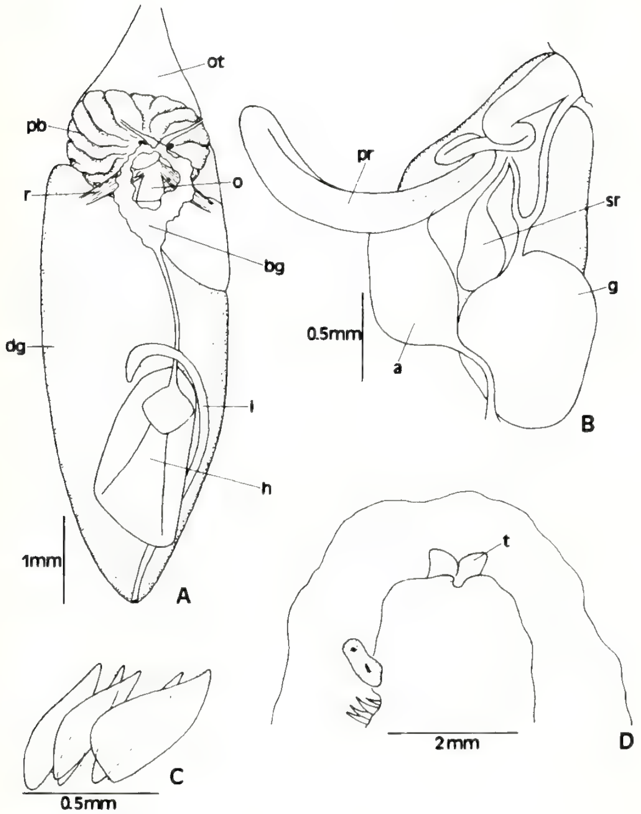

Fig. 8. *Reticulidia gofasi,* paratype from Atlantis Bank (MNHN). **A.** Dorsal view of the anatomy. **B.** Genital system. **C.** Detail of the branchial leaves. **D.** Ventral view of preserved specimen showing the oral tentacles. a, ampulla; bg, blood gland; dg, digestive gland; g, gametolytic gland; h, heart; i, intestine; o, esophagus; ot, oral tube; pb, pharyngeal bulb; pr, prostate; r, retractor muscles; sr, seminal receptacle; t, oral tentacles.

several discs, around a hole through which the short esophagus emerges (Figs. 4C, 8A). Also, two large retractor muscles insert on the pharyngeal bulb at both sides of the esophagus.

The genital system (Fig. 8B) presents an ampulla larger than the seminal receptacle. The prostatic portion of the deferent duct is quite short, with only one fold, and connects with the deferent portion by a short and thin duct.

Geographical Range

Reticulidia gofasi is only known in the Meteor Group seamounts (Atlantis Bank), Lusitanian seamounts (Josephine Bank), and the Azores (slope of Terceira) (Fig. 5).

Discussion

The structure of the pharyngeal bulb of *Reticulidia gofasi*, with several discs and two large retractor muscles, is identical to that described by Brunckhorst (1990a, 1993) for the genus *Reticulidia*. However, the dorsal external morphology of the Indo-Pacific species of this genus, *Reticulidia halgerda* Brunckhorst and Burn, 1990, and *Reticulidia fungia* Brunckhorst and Gosliner, 1993, with bright colors and dorsal ridges, is very different from our species, pale yellow with conical tubercles. The color differences of *R. gofasi* with the other species of the genus could be connected with the bathyal habitat of the former; the same occurs for other deep water phyllidiids such as *Fryeria bayi, Phyllidiopsis berghi*, and *P. boucheti.*

In the external morphology and anatomy, *Reticulidia gofasi* is clearly different from the other Atlantic phyllidiid species. The morphology of the oral tentacles, the shape and distribution of the dorsal tubercles, and the structure of the digestive system are very distinctive characters of this species.

Etymology

The name *Reticulidia gofasi* is in honor of our friend and colleague, Dr. Serge Gofas (MNHN), who collected and made available to us most of the material of this species.

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We are very grateful to Dr. Serge Gofas (MNHN), Dr. Philippe Bouchet (MNHN), Dr. Terrence Gosliner (California Academy of Sciences, San Francisco), and Gilianne Brodie (James Cook University, Australia) for constructive comments on the manuscript. We also acknowledge the valuble comments of Editor Dr. R. Toll and two anonymous reviewers. Material studied has been provided by Dr. Alan Kabat (USNM), Dr. Juan J. Bacallado (Museo Insular de Ciencias Naturales, Tenerife, Spain), and Dr. José Espinosa (Instituto de Oceanología, Havana, Cuba).

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LITERATURE CITED

Bergh, R. 1890. Report on the results of dredging, under the supervision of Alexander Agassiz, in the Gulf of Mexico (1877-78), and in the Caribbean Sea (1879-80), by the U. S. Coast Survey Steamer "Blake", Lieut.-Commander C. D. Sigsbee, U. S. N., and Commander J. R. Bartlett, U. S. N., Commanding. 32. Report on the nudibranchs. *Bulletin of the Museum of Comparative Zoology* 19(3):155-181.

Bouchet, P. 1977. Opisthobranches de profondeur de l'Océan Atlantique: II, Notaspidea et Nudibranchiata. *Journal of Molluscan Studies* 43:28-66.

Brunckhorst, D. J. 1990a. Description of a new genus and species belonging to the family Phyllidiidae (Nudibranchia, Doridoidea). *Journal of Molluscan Studies* 56:567-576.

Brunckhorst, D. J. 1990b. Description of a new species of *Phyllidiopsis* Bergh (Nudibranchia, Doridoidea, Phyllidiidae) from the tropical western Pacific, with comments on the Atlantic species. *Journal of Molluscan Studies* 56:577-584.

Brunckhorst, D. J. 1993. The systematics and phylogeny of phyllidiid nudibranchs (Doridoidea). *Records of the Australian Museum*, supplement 16:1-107.

Eliot, C. 1903a. On some nudibranchs from East Africa and Zanzibar, 2. *Proceedings of the Zoological Society of London* 1903:250-255.

Eliot, C. 1903b. Nudibranchiata, with some remarks on the families and genera and description of a new genus, *Doridomorpha. Fauna and Geography of the Maldive and Laccadive Archipelagoes* 2(1):540-573.

Gosliner, T. and D. Behrens. 1988. A review of the generic divisions within the Phyllidiidae with the description of a new species of *Phyllidiopsis* (Nudibranchia: Phyllidiidae) from the Pacific coast of North America. *The Veliger* 30(3):305-314.

Marcus, Ev. and Er. Marcus. 1962. Opisthobranchs from Florida and the Virgin Islands. *Bulletin of Marine Science of the Gulf and Caribbean* 12(3):450-488.

Marcus, Ev. and Er. Marcus. 1967. Tropical American opisthobranchs. *Studies in Tropical Oceanography, Miami* 6:1-137.

Meyer, K. B. 1977. Dorid nudibranchs of the Caribbean coast of the Panama Canal Zone. *Bulletin of Marine Science of the Gulf and Caribbean* 27(2):299-207.

Thompson, T. E. 1980. Jamaican opisthobranch molluscs II. *Journal of Molluscan Studies* 46:74-99.

Vayssière, A. 1902a. Sur les opisthobranches recueillis en 1883 par l'expedition du "Talisman." *Comptes Rendus Hebdomadaries des Sciences et Memories de la Societé de Biologie et de ses Filiales* 134:296-297.

Vayssière, A. 1902b. Opisthobranches du "Talisman" campagne de 1883. *In: Expédition scientifique du "Travailleur" et du "Talisman" pendant les années 1880-1883*, pp. 221-271. Ministére de l'Instruction Publique, Paris.

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Fargoa bartschi (Winkley, 1909): a little-known Atlantic and Gulf coast American odostomian (Pyramidellidae) and its generic relationships

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Abstract: The shell and animal morphology and coloration of the pyramidellid *Fargoa bartschi* (Winkley, 1909) are described. *F. bartschi* is newly recorded in shallow water (maximum known depth *ca.* 30 m) from Massachusetts to Texas. In Massachusetts its host is the serpulid polychaete *Hydroides dianthus* (Verrill, 1873). *F. bartschi* and its congener *F. dianthophila* (Wells and Wells, 1961) occur on the same host and sometimes the same worm. The egg of *F. bartschi* is *ca.* 58 µm in diameter and the species is "planktotrophic" despite its protoconch suggesting lecithotrophy. The two species are greatly different in size and shell sculpture but are shown to be closely related. It is argued that shell sculpture is a poor generic character in pyramidellids.

Key words: *Fargoa*, Pyramidellidae, generic relationships, zoogeography, *Hydroides*

Pyramidellid systematics is notoriously chaotic. Even so, the low intertidal and shallow subtidal zones of a malacologically well-explored place like Massachusetts seem an unlikely place for the discovery in the late twentieth century of a 4 mm-long pyramidellid. Yet this is the case with *Fargoa bartschi*, although the species is shown to have been first validly named in 1909 and to have undergone nomenclatural turmoil since then. It is not listed in the American marine malacological "Bible" by Abbott (1974). *F. bartschi* is here shown to have a typically Virginian and Carolinian distribution, ranging to Texas (probably omitting southeastern Florida).

The genus *Fargoa* was described and named by Bartsch (1955). It was first applied by Robertson (1978) to a variously sculptured genus found to have shell-attached spermatophores and other distinctive animal characters. *Fargoa* parasitizes serpulids and *Boonea* Robertson, 1978 mollusks (Robertson and Mau-Lastovicka, 1979). *Boonea* and *Fargoa* have undergone shell sculptural divergences and convergences between closely related species (Robertson, 1978).

Bartsch (1909), who studied the New England pyramidellids, did not mention *"Odostomia" bartschi* (Winkley, 1909) because this was published five months later. Bartsch did not illustrate it under another name — he probably had not seen it or dismissed it as juvenile. Abbott (1974) did not mention or illustrate *O. bartschi* even as a synonym. He may, though, have used for it either the European species name *O. conoidea* (Brocchi, 1814) or *O. dealbata* (Stimpson, 1851). Abbott considered *O. modesta* Stimpson, 1851b, a probable synonym of *O. conoidea*. This is here considered doubtful and *O. modesta* is shown to be a primary homonym and *O. dealbata* a *nomen dubium.* Andrews (1971) did not mention the species from Texas. Thus *"O." bartschi* is a nearly forgotten name, having been used only once between 1916 and 1978, when I first transferred it to *Fargoa*. The binomen *Fargoa bartschi* has been used in only two other papers since then (Robertson and Mau-Lastovicka, 1979; Robertson, 1985).

This non-cladistic paper enumerates the characters distinguishing *Fargoa*, especially external morphologies, coloration, and host preferences. Then *F. bartschi,* one of the least known species, with a rather characterless shell, is described from shell and animal data. It is compared and contrasted with *F. dianthophila*, which is shown to be closely related despite its smaller size and greatly dissimilar shell sculpture.

MATERIALS AND METHODS

This study is based on a few living animals from Massachusetts, North Carolina, and Texas, and numerous shells (mostly from Massachusetts) in the Museum of Comparative Zoology (MCZ), The Academy of Natural Sciences of Philadelphia (ANSP), and the National Museum of Natural History (USNM); Smithsonian Institution, Washington, D.C. Specimens were kept alive (and produced spermatophores) in plastic petri dishes. The sea water was changed daily. Life history observations are based mainly on specimens collected at Woods Hole, Massachusetts.

GENERIC DESCRIPTION

Family Pyramidellidae
Genus *Fargoa* Bartsch, 1955

Type species (by original designation): *Fargoa calesi* Bartsch, 1955 ("Pliocene" or Pleistocene) = *Odostomia bushiana* Bartsch, 1909, not *O. bushiana* (Jeffreys, 1884).

Shells up to *ca.* 5 mm long, with reticulated nodes, with or without single spiral cord, or entirely smooth. Protoconch slightly or not tilted, less than one whorl, not detectably heterostrophic. Operculum amber, transparent except for opaque white spiral middle sector, radially wrinkled, with sinus for columellar plica (Figs. 7-8). Body cream-white with various patterns and colors. Foot slit medially (the pedal mucous pore is here), but slit not extending to posterior end (Fig. 9). Median anterior of foot cleft (Figs. 9-10). Pigmented mantle organ (seen through shell) conspicuous, yellow and brown or veneered dark brick-red. Fully everted proboscis 1 1/2 to 2 times shell length. Spermatophore with a bulb and "neck" leading from it (Figs. 11-13), becoming shell-attached in a characteristic position. Ecoparasitic on *Hydroides* and *Eupomatus* (serpulid polychaetes) (Wells and Wells, 1969).

SYNONYMIES OF THE TWO SPECIES COMPARED

Fargoa bartschi (Winkley, 1909)
(Figs. 1-15, Table 1)

Chemnitzia modesta Stimpson, 1851a: 16 ("on stones drawn up by the fishermen from about thirty fathoms [55 m], on St. George's Bank"). — Not *C. modesta* d'Orbigny, 1842: 222-223, pl. 16, figs. 22-24 [a Jamaican *Turbonilla*]. — Stimpson, 1851c: 41-42, "This species is more angular than *C. bisuturalis* [Say, 1822], and has no revolving line just below the suture as in that shell. It is very like the British *O. unidentata*" (Montagu, 1803) [*Turbo*]. "The Coralline zone..." Holotype probably destroyed in the Chicago fire of 1871 (Dall, 1888). *Odostomia unidentata* is a European species erroneously recorded from North Carolina to Florida by Johnson (1934). Judging by Fretter *et al.* (1986), true *O. unidentata* is less like *Fargoa bartschi* than *O. acuta* Jeffreys, 1848 (see below).

Odostomia modesta (Stimpson), Gould, 1870: 327, fig. 596. — Tryon, 1873: 67, pl. 10, fig. 117. — Blaney, 1904: 34, "Frenchman's Bay, Maine." — "*O. modesta* Verrill" Bush, 1909: 482, fig. 8, "Eastport. Me. [Maine]. Mass.[achusetts], 6-115 fath." [11-210 m]. — Johnson, 1915: 100 "ME. [Maine] - Frenchman's Bay, 6-8 fathoms [11-15 m]. (Blaney). MASS.[achusetts] - Woods Hole; south of Martha's Vineyard, 115 fathoms [210 m]; Duxbury." — Johnson, 1934: 91, "Eastport, Me., 6-115 fath." [11-210 m]. — Robertson, 1967: 369 (spermatophores). — Odé and Speers, 1972: 16. — Mayhew and Cole, 1995: 110, north of Cape Cod.

Odostomia (Odostomia) modesta Bartsch, 1909: 108, pl. 13, fig. 50, is not *O. modesta* (Stimpson), but *O. gibbosa* Bush, 1909: 482.

Pyramidella (Sulcorinella) bartschi Winkley, 1909: 39-40, 1 fig., "Woods Holl, Mass.[achusetts]." Holotype, MCZ 32810 (Robertson, 1978, fig. 8; fig. 2). Winkley illustrated a "moderately deep [peripheral] sulcus, which is bounded on each side by a slender raised thread." He exaggerated the rare, slightly raised spiral bands.

Odostomia (Evalea) bartschi (Winkley), Winkley, 1912: 54. — Johnson, 1915: 98, "... Chatham; Duxbury; North Falmouth."

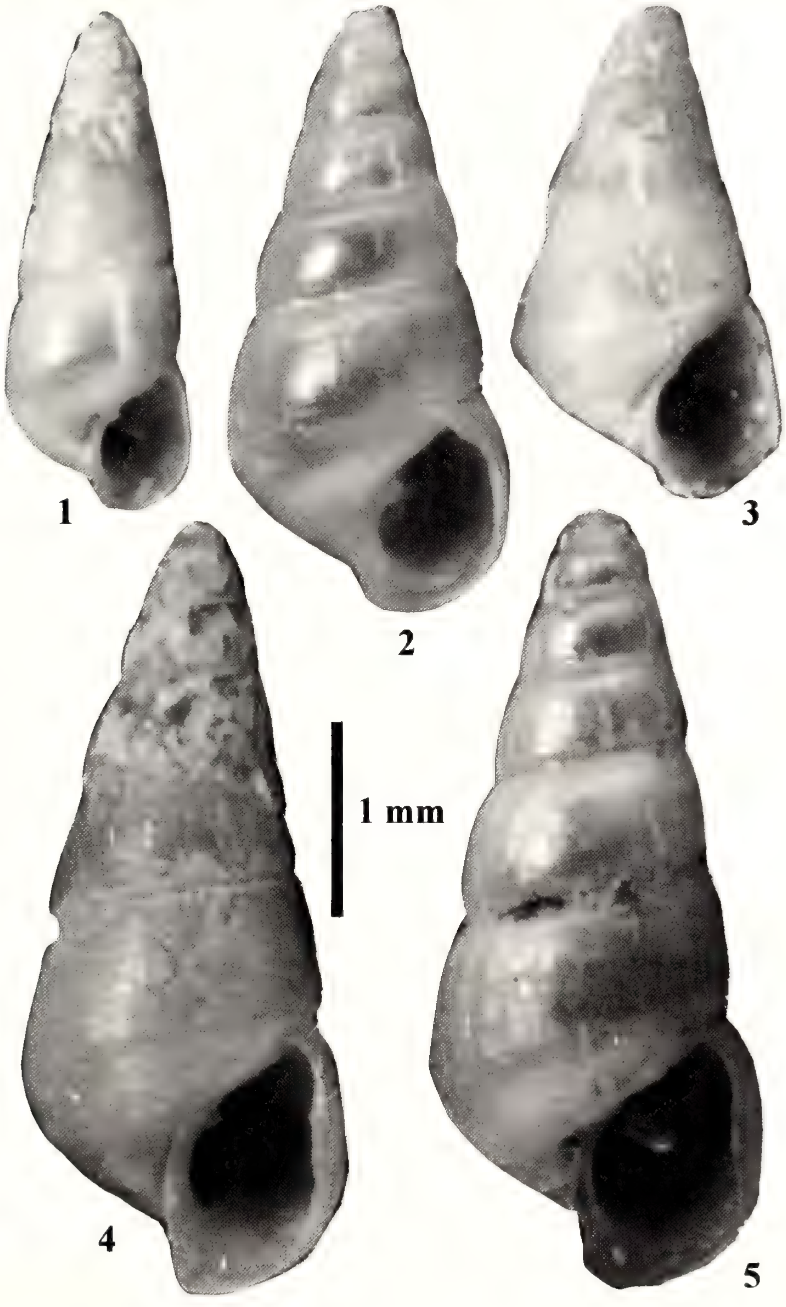
Odostomia bartschi (Winkley), Winkley, 1916: 110, "Nantucket."

Eulimastoma bartschi (Winkley), Odé and Speers, 1972: 12, 14-15, "Port Aransas" [Texas]. — Odé, 1994: 17, 20, 26 ["c.f." *bartschi*], 32, fig. 11.

Fargoa bartschi (Winkley), Robertson, 1978: 376-377, figs. 8 (holotype), 72-76. -- Robertson and Mau-Lastovicka, 1979: 321-332. — Robertson, 1985: 1-6, fig. 1 (histological cross-section of mantle cavity).

Other names misapplied to *F. bartschi*

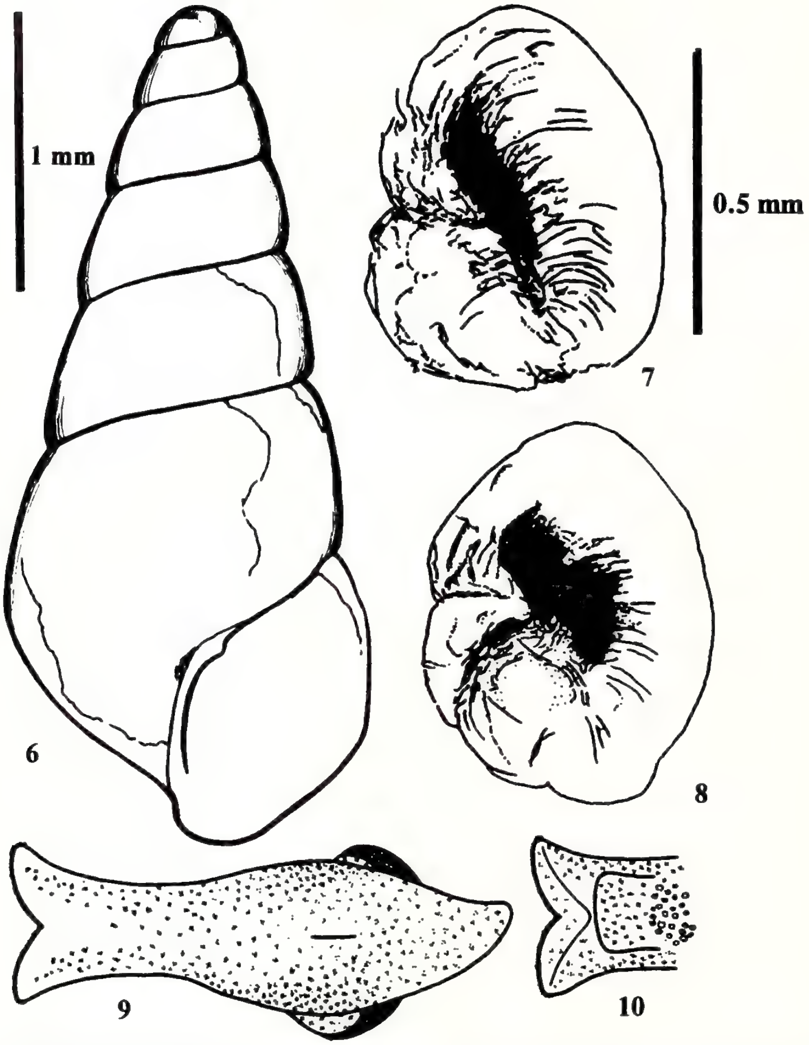
Odostomia conoidea (Brocchi, 1814: 660, pl. 16, fig. 2) [*Turbo*]. — Johnson (1934: 91, as *O. conidea*), without explanation, recorded this from "North Carolina to the Gulf of Mexico." Abbott (1974: 292, species nos. 2475 and 3478) recorded *O. modesta* as a probable synonym of this European species, which he recorded from "Eastport, Maine to off Martha's Vineyard, Massachusetts, 6 to 115 fms. [11-210 m] ... North Carolina to west Florida ... 14-24 fathoms [26-44 m], ... and British Isles. Mediterranean." No pyramidellid is yet known to be amphi-Atlantic and *Fargoa bartschi* does not resemble *O. conoidea* of authors. This is larger (up to 5.7 mm long), has a tiny heterostrophic protoconch tilted at about 90° (Thorson, 1946: fig. 116C?), more evenly rounded whorls, a more swollen spire, a stronger columellar



Figs. 1-5. *Fargoa bartschi*. Shells from Massachusetts. 1. Unusually narrow shell (MCZ 33751). 2. Holotype of *Pyramidella (Sulcorinella) bartschi* (MCZ 32810). 3. Unusually wide shell (MCZ 33745). 4-5. Two of the largest shells.

fold, and the outer lip is internally lirate (nine ANSP lots were studied). *O. conoidea* was illustrated by Fretter *et al.* (1986: fig. 427). Brocchi's (1814) original description and figure of *Turbo conoideus* show an Italian Tertiary shell more similar to *Fargoa bartschi*, but on the basis of the tiny figure, distribution, and age, they are unlikely to be conspecific. Following Abbott (1974), Miller (1983) recorded *O. conoidea* from the "Wassaw Sound area, coastal Georgia" [U. S. A.]; presumably he had *F. bartschi*.

Chemnitzia dealbata Stimpson, 1851b: 114, "Boston Harbor." –– Stimpson, 1851c: 41, "Dredged in Boston Harbor, in 3 f. [5.5 m] on a shelly bottom." The holotype, like that of *C. modesta*, probably was destroyed in the Chicago fire of 1871. The parts of Stimpson's Latin description that seem not to agree



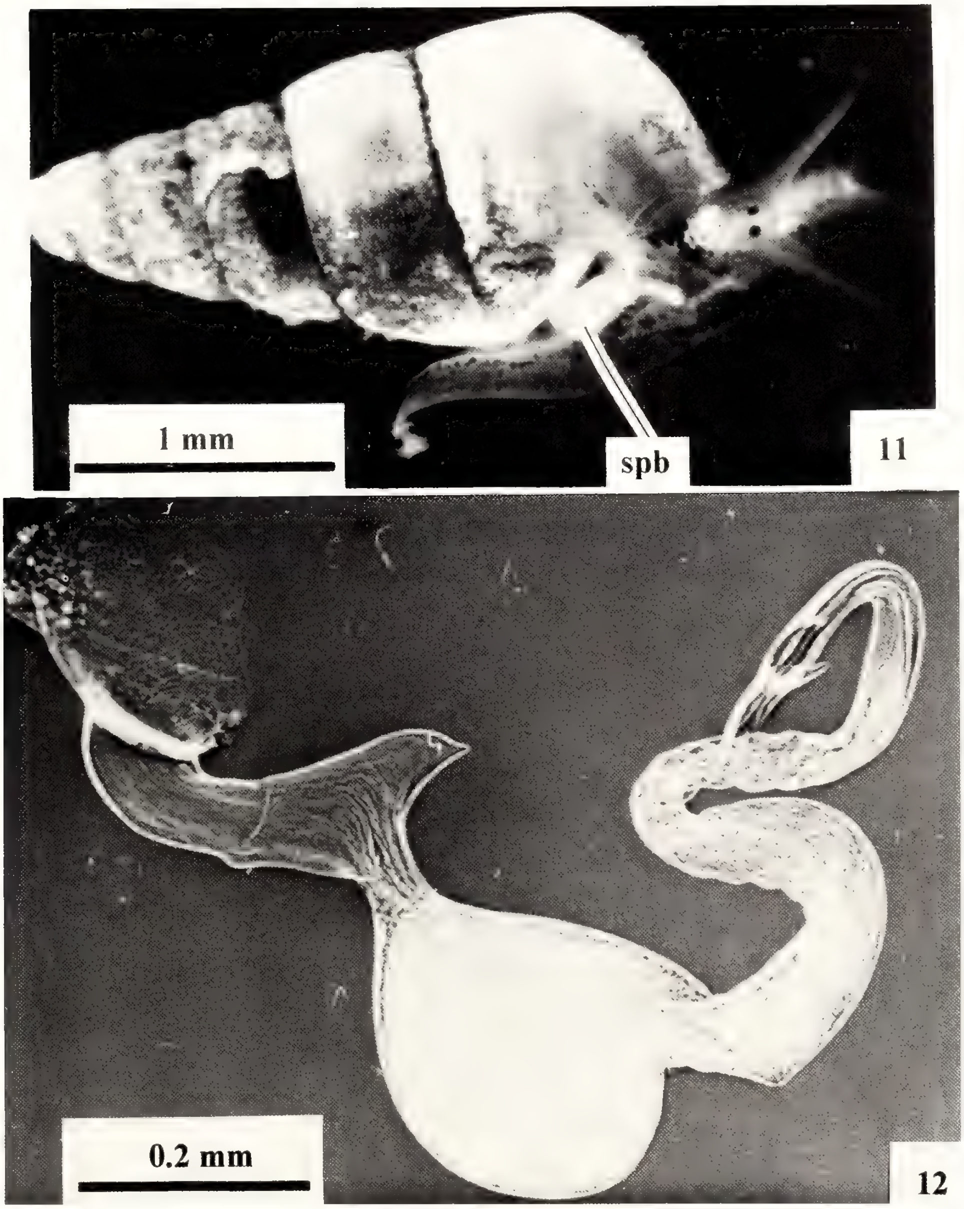
Figs. 6-10. *Fargoa bartschi.* 6. Outline of largest observed shell. 7-8. Opercula, showing variation (opaque white shown as black). 9. Ventral view of foot (diagrammatic), showing median slit. 10. Dorsal view of propodium and mentum (diagrammatic).

with *Fargoa bartschi* are (my translation): "shell ovate-conic, pellucid and thin, with rather convex whorls, and a ... small, inconspicuous plica." Stimpson (1851c: 41) also stated that "it is broader than *C. bisuturalis*, has not so sharp an apex, and wants the revolving line."

Odostomia dealbata (Stimpson), Gould, 1870: 327, fig. 595. In his editorial preface, Binney (in Gould) acknowledged his indebtedness to Dr. W. Stimpson for the "valuable assistance in preparing the work." Thus it is likely that Stimpson approved of the treatment of his two species. Gould's fig. 595 shows a shell highly dissimilar to *Odostomia modesta* (his fig. 596). — Tryon, 1873: 67, pl. 10, fig. 116. — Bush, 1909: 482, fig. 6 (typical form, not as in Gould). Bush unnecessarily renamed *O. dealbata* Gould (not Stimpson, 1851b) *O. gouldii.* — Johnson, 1915: 100, "MASS.[achusetts] - Boston Harbor, 3 fathoms [5 m]; Duxbury. CONN.[ecticut]. Near New Haven (Perkins)." — Johnson, 1934: 91. — Odé and Speers, 1972: 16. — Abbott, 1974: 292, species no. 3475. — Merrill *et al.*, 1978: 38.

Fargoa dianthophila (Wells and Wells, 1961)
(Figs. 16-18, Table 1)

Odostomia (Chrysallida) dianthophila Wells and Wells, 1961: 152, figs. 1-3, "Beaufort, North Carolina" (host). — Odé, 1967: 24, Palacios, Texas. — Odé, 1968: 17, Matagorda Beach, Texas. — Roberge, 1968: iii, Massachusetts (host). — [Odé], 1969: 36.



Figs. 11-12. *Fargoa bartschi* spermatophores. 11. *In situ* on living animal, with the spermatophore bulb (spb) indicated; the structure is attached posteriorly to the last shell whorl. 12. Detached structure showing (from left to right) the holdfast, leg, bulb, neck, crook, and barb.

— Wells and Wells, 1969: 109-110 (hosts). — Odé, 1971: 89, fig. 1, San Luis Pass, Texas, "Best placed in the genus *Fargoa*."

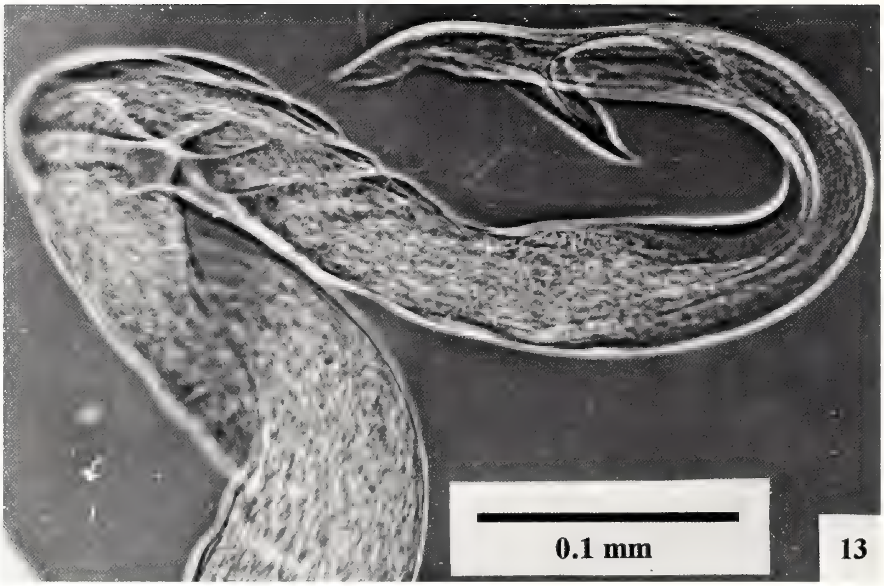
Fargoa dianthophila (Wells and Wells, 1961), Odé and Speers, 1972: 10-11. — Robertson, 1978: 373, figs. 6, 68-70 (spermatophores). — Odé, 1993: 29, Texas.

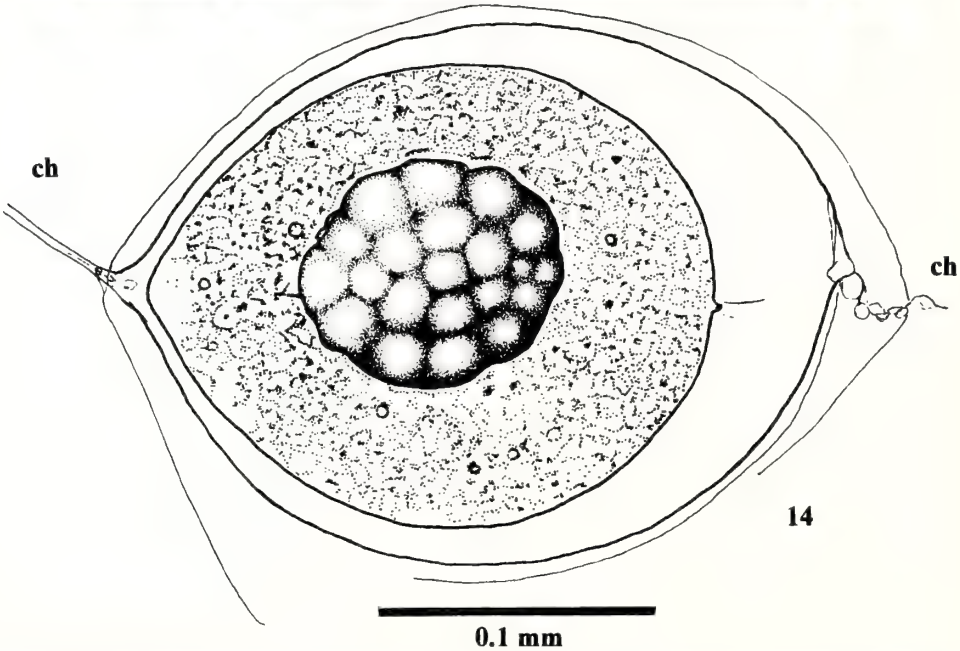
Hosts: Ectoparasitic on *Hydroides dianthus* (Verrill, 1873) in North Carolina and Massachusetts and other species of *Hydroides* and *Eupomatus* farther south and into the Gulf of Mexico.

RESULTS: DESCRIPTION OF *FARGOA BARTSCHI*

SHELL DESCRIPTION

Shell thin, semitransparent except where corroded and opaque. Largest shell 4.4 mm long, 1.8 mm wide. Protoconch nearly isostrophic, smooth, *ca.* 0.9 whorl, slightly flattened and tilted, 0.21-0.23 mm wide (N = 5). Up to *ca.* 5.6 teleoconch whorls. Traces of sculpture on first teleoconch whorl (rarely weak axial ribs). Spire high; spire angle 30-35° (Figs. 1-6). Whorls slightly inflated.





Figs. 13-14. *Fargoa bartschi.* 13. Distal end of spermatophore, showing neck (in part), crook, barb, and terminal pore (*ca.* 10 µm in diameter). Note euspermatozoa near barb and pore. 14. Many-celled embryo developing inside the cocoon (inside the gelatinous egg mass). Chalazae (ch) connect adjacent cocoons.

Periphery slightly angled or rounded, rarely with one or two narrow or one wide raised cords or bands (especially on juveniles). Suture moderately impressed, whorl attached just below periphery. Surface smooth except for very faint spiral threads. No incised spiral line subsuturally or elsewhere. Moderately wide anal sinus; outer lip otherwise nearly orthocline. Narrow umbilicus present. One prominent plica on columella. Aperture ovate except for columellar plica. Lower lip not confluent with basal outline. Palatal wall not lirate. Anterior end roundly quadrate. White to pale grey or pale amber, or pale orange-brown. Amber or amber-brown except where corroded (then off-white).

Corroded apices, repaired breaks, cessations of growth, and scratches and scrapes are frequent; the shells are chalky where eroded or corroded. Foraminiferans commonly are epizoic. The umbilicus commonly is occluded

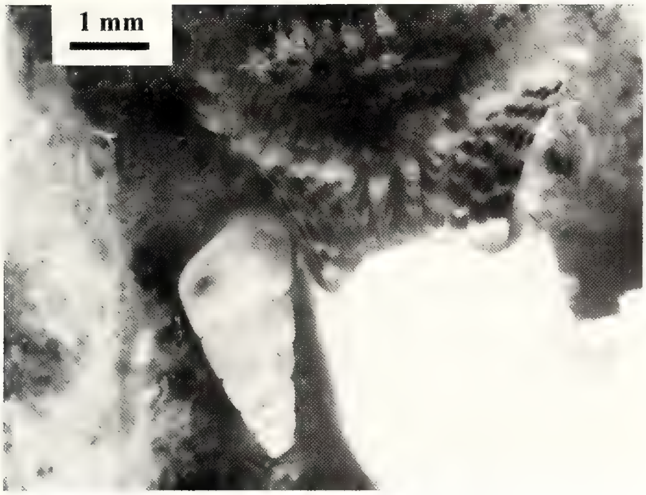


Fig. 15. *Fargoa bartschi* next to the tube of its partially extended tubeworm host *Hydroides dianthus* (Woods Hole). Note the conspicuous pigmented mantle organ.

with sand. The shell is pale orange-brown where stained by iron oxide. The portion of shell freshly grown in the new year is distinguishable from the more eroded shell produced the preceding year.

ANIMAL CHARACTERS

Digestive gland grey, heavily speckled with black, brown, grey, or greenish, visible through spire. Pigmented mantle organ (PMO) elongated ovate, bright yellow veneered with dark purplish brown or variegated dark brick-red; PMO conspicuous, visible under low magnification through penultimate whorl of shell (sometimes retained in dried, live-collected specimens). Ground color pale grey, translucent. Statocysts very faintly visible below eyes through foot or laterally, diameter 10-13 µm. Genital pore on right side of foot, opposite basal mentum. Excurrent siphon projecting considerably; juxtaposed flap present. Superficial brown spots behind head; golden yellow, yellow brown, orangish or greenish spots elsewhere (on mentum, tentacles, excurrent siphon, and foot). Deeper white granules posterior to mentum and eyes, under operculum. Tentacles cream or yellow, with distal nodes. Foot relatively elongated (Fig. 9). Pedal pore with short slit anteriorly and posteriorly (not extending to either end of foot).

LIFE HISTORY

The animals are scarce (but were somehow obtained by W. F. Clapp and H. W. Winkley in quantity; see Locality Records below). During the daytime they live singly and in pairs beneath loose rocks and stones, 0-1 m deep at low spring tide. *F. bartschi* is ectoparasitic on *Hydroides dianthus*, living beside the aperture (Fig. 15) (adults are too large to ride in and out on the worm's operculum or to turn around inside of the worm's tube as does *Fargoa dianthophila*). Occasionally, both species occur on the same individual worm. Feeding was observed in the laboratory only once (the animal is very finicky); the proboscis tip pierced one of the worm's gill filaments. The species is annual, breeding in summer. Prior to spermatophore transfer, the donor crawls "meticulously" over the shells of the recipients, touching these with their propodia and mentums. The egg masses are gelatinous, thick C-shaped; chalazae are present; there are fewer eggs per mass than in *Boonea*. The eggs are small, diameter *ca.* 58 µm, 15-246 per mass (mean 122; N = 5). The species is "planktotrophic" despite its direct development-type protoconch (Thorson, 1946). Hatching veligers were seen at an early stage of development, and seen swimming. Veliger feeding was not observed. Maximum diameters of the newly hatched veliger shells were 129-135 µm (mean 132.5; N = 10). The veliger shells are nearly isostrophic. Newly settled *Fargoa bartschi* and *F. dianthophila* are indistinguishable, sometimes clustering on the same worm. They become distinguishable when the latter begins to develop sculpture and distinctive body colors. The 0.2 mm protoconch diameter reflects growth in the plankton.

North Carolinian shells are mature at as small a shell length as 1.0 mm, and the spermatophores are correspondingly smaller than those from Massachusetts

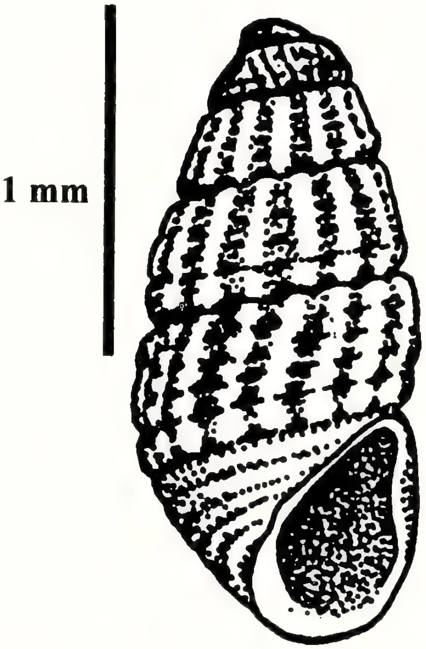


Fig. 16. *Fargoa dianthophila.* Shell 1.8 mm long; Beaufort, North Carolina; holotype? The species has a columellar plica that does not show in this view (from Wells and Wells, 1961).

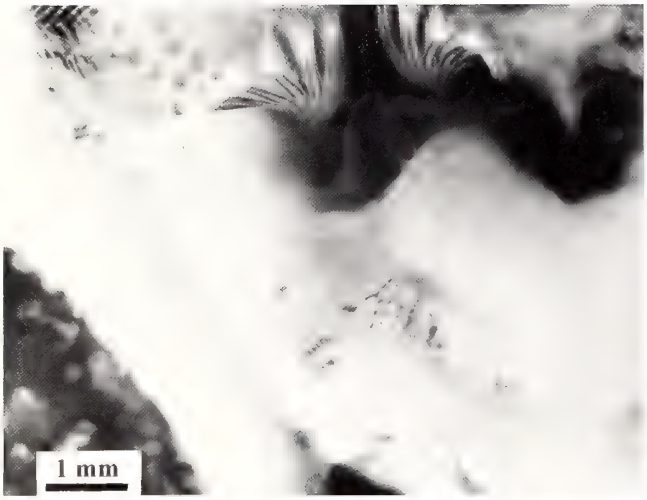


Fig. 17. Pair of *Fargoa dianthophila* on the tube of its host *Hydroides dianthus* (Woods Hole). The animal on the right is feeding. Note the pigmented mantle organ on each animal.

(Robertson, 1978). No Texan spermatophores have been seen, but the shells are small there also.

GEOGRAPHICAL RANGE AND BATHYMETRY

Known from Cape Cod Bay, Massachusetts, to southern Texas, a typically Virginian and Carolinian distribution (Coomans, 1962). Possibly occurs north to Maine (Blaney, 1904; Johnson, 1915, both as *Odostomia modesta*), but these records may have been unincised *Boonea bisuturalis*. I have no Floridian records so far, but *Fargoa bartschi* can be expected there in the north. Occurs in the low intertidal zone down to *ca.* 30 m (New Jersey), very doubtfully to 210 m (Bush, 1909, as *O. modesta*).

LOCALITY RECORDS

Massachusetts (north of Cape Cod): Duxbury, 360 live spms, coll. W. F. Clapp, July 7 - Sept. 15, 1913, July 28, 1914; Bridge Flat (USNM 503903, 503902, 503905), flat near high pines (USNM 503927, 503914), Lucas Flat (USNM 503900), Winsor Flat (USNM 503898), flat opposite Bridge Flat (USNM 503909), flat opposite Winsor[s] (USNM 503907), above bridge, right hand side going up (USNM 503906), east end flat opposite Winsor Flat (USNM 503931), and Two Rock Flat (USNM 503920). — Duxbury (unlocalized), 9 live spms, coll. W. F. Clapp (MCZ 33742, via H. W. Winkley, 1918). — Saquish Cove, Plymouth, dredged 2-3 m, 1 live spm, coll. R. K. Smith and W. F. Clapp, August 1910 (MCZ 14158; Fig. 5).

Massachusetts (south of Cape Cod): Chatham, 23 live spms, coll. H. W. Winkley (MCZ 33753). –– Nantucket, 50 live spms, coll. H. W. Winkley (MCZ 33745; Fig. 3). — Woods Hole (topotypes), 69 live spms, coll. H. W. Winkley (MCZ 33743, 33750, 32810 holotype; Fig. 2). Also coll. Winkley: Eel Pond, 6 spms (labelled *Odostomia (Evalea) bartschi*) (USNM 250646); N. W. Gutter (MCZ 33744); N. W. Souther (?), 26 live spms (MCZ 33751; Fig. 1). — Shore opposite Flume Pond, south of Gunning Point, 4 km NNE of Woods Hole, and northern end of Quissett Harbor, 3 km NE of Woods Hole, coll. R. Robertson (spermatophores studied). — Gay Head, Martha's Vineyard, 1 live spm, coll. H. W. Winkley (MCZ 32811).

New Jersey: *ca.* 32 km SE of Absecon Light, Atlantic City, 39.20°N, 74.27°W, *ca.* 30 m, 6 freshly dead spms, coll. by Lewis Woolman on *Placopecten magellanicus* (Gmelin, 1791) (with *Hydroides* on it?) (ANSP 19978).

North Carolina: *ca.* 32 km off Beaufort, 1 live spm on sand, 20 m, coll. by C. Thiriot-Quiévreux, July 1979 (ANSP 350327). Also Wreck Point, Cape Lookout, 16 km of SE of Beaufort, and west side of Banks Channel, Wrightsville Beach, 14 km ESE of Wilmington, all live coll., R. Robertson (spermatophores studied).

South Carolina: Bulls Bay and Bulls Isl., Charleston Co., coll. T. L. Laavy (his collection).

Texas: Aransas Bay, off Rockport, 28.02°N, 97.04°W, dredged in shallow water off grass flat, 10 live juveniles, coll. Anne Speers, September 1961 (ANSP 280273). Also several other Texas localities, coll. Constance E. Boone.

SALINITY AND TEMPERATURE TOLERANCES

Stenohaline. Four live-collected specimens from Hadley Harbor, Woods Hole, in the Systematics Ecology Program collection (Marine Biological Laboratory) were found in salinities ranging from 31.93 - 32.17 ppt. The

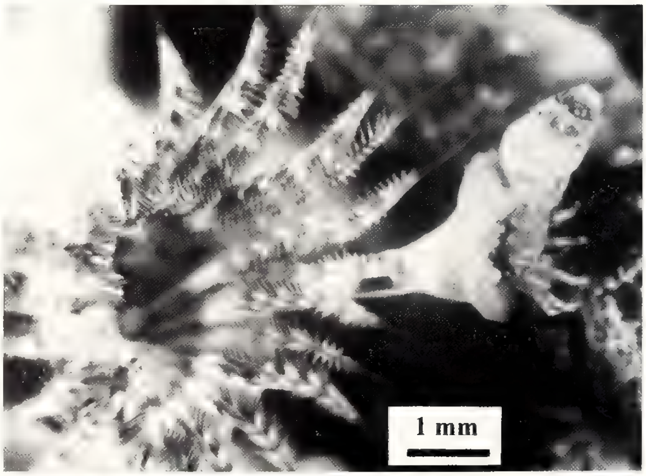


Fig. 18. *Fargoa dianthophila* on its extended *Hydroides* host's operculum, where it can ride in and out of the tube (Woods Hole).

localities listed above also suggest that the species does not enter estuaries.

Like any shallow subtidal species in the Virginian faunal Province, this one must be eurythermal. At least in the north, there is winter cessation of growth.

DISCUSSION AND CONCLUSIONS

Fargoa bartschi and *F. dianthophila* are closely related congeneric species (Table 1) that have undergone evolutionary divergence in shell sculpture. *F. bartschi* has a nearly smooth shell up to 4.4 mm long, and *F. dianthophila* has a noded shell only up to 1.8 mm long. The former lives outside the tubes of its serpulid host, and the latter can be pulled inside the tubes on the worm's "operculum" (Fig. 17). The two fargoas can parasitize the same individual worm. The two species have similar life histories: at least in Massachusetts, both breed in summer, have small or tiny eggs, "planktotrophic" veligers, and are annual. The protoconchs are of the same size and morphology. The young postlarval pigmented mantle organs are of the same colors (yellow and brown) (Robertson, 1985). In the fall, the benthic postlarvae (already clustering around *Hydroides*) are indistinguishable until sculpture begins to develop on the shells of *F. dianthophila*.

Systematists have considered shell sculptures of paramount importance as generic characters. This belief was called into question by Robertson (1978) and here. Among the sympatric northeastern American odostomians (*Odostomia*-like pyramidellids), the shells of which were illustrated by Robertson (1978), the smooth *Fargoa bartschi* shell most closely resembles the unincised form of *Boonea bisuturalis* (not illustrated). *F. gibbosa* (Bush, 1909) is also smooth, but much more inflated. *F. dianthophila*, with its noded shell, resembles small *B. seminuda* (C. B. Adams, 1839) and (to a lesser degree) *F. calesi* (which has nodes and one subsutural spiral cord). Thus there have been evolutionary convergences as well as divergences among some of these American odostomians. There is as yet no known *Fargoa* resembling the strongly spirally sculptured *Boonea, B. impressa* (Say, 1822).

The unreliability of pyramidellid generic shell characters contrasts with them as species characters, which are generally reliable. Even among these, though, there has been some documentation of intraspecific shell variability among odostomians (Winkley, 1901; Scheltema, 1965; Porter, 1976; LaFollette, 1977; Porter *et al.*, 1979).

There are Recent species in other geographical areas with teleoconchs like those of *Fargoa bartschi*. Examples from the world pyramidellid literature include:

Odostomia acuta Jeffreys, 1848. Fretter *et al.*, 1986: fig. 423; van Aartsen, 1987: fig. 15. Europe.
O. angularis Dall and Bartsch, 1907; Dall and Bartsch, 1909: pl. 24, fig. 6. Alaska to Monterey Bay, California.
O. bedoti (Hornung and Mermod, 1924). Saurin, 1958: pl. 1, fig. 4; 1959: pl. 2, fig. 19. Vietnam.
O. canaliculata (C. B. Adams, 1850). Clench and Turner, 1950: pl. 40, fig. 3. Jamaica. Argentina? (Castellanos, 1982: figs. 20, 20A).
Parodostomia compta (Brazier, 1877) [originally *Odostomia compta*]. Laseron, 1959: fig. 48. Northern and Western Australia.
O. puelchana Castellanos, 1982: fig. 16. Argentina.
O. shimosensis Yokoyama, 1922. Nomura, 1937: figs. 22a, 22b. Japan.

Note that all seven of these species have been placed in the "genus" *Odostomia* Fleming, 1817. It is a thesis of this paper that this is a heterogeneous array of allopatric species that have come superficially to resemble each other by convergence, all acquiring smooth teleoconchs, commonly with a trace of peripheral angulation.

Table 1. Similarities and differences between *Fargoa bartschi* and *F. dianthophila.*

Similarities (many are generic characters)

1. Protoconch with no heterostrophy, slightly tilted; larvae nearly isostrophic.
2. Young postlarval shell nearly smooth.
3. Columellar plica present.
4. Anterior end foot cleft (Figs. 9-10); adapted to position spermatophores?
5. Underside of foot with pore or slit not extending to posterior end.
6. Larval pigmented mantle organ yellow and brown.
7. Host-specific to serpulid polychaetes (*Hydroides dianthus* near Woods Hole).
8. Everted proboscis *ca.* twice shell length.
9. Digestive gland grey black.
10. Spermatophores shell-attached.
11. Veligers "planktotrophic".
12. Operculum with opaque white spiral sector (Figs. 7-8)

Differences

F. bartschi	*F. dianthophila*
13A. Shell up to 4.4 mm long, does not enter *Hydroides* tubes.	13B. Shell up to 1.8 mm long, does enter *Hydroides* tubes.
14A. Adult shell nearly smooth.	14B. Adult shell noded.
15A. Adult pigmented mantle organ dark red-brown.	15B. Adult pigmented mantle organ yellow and brown.
16A. Underside of foot with slit (Fig. 9).	16B. Underside of foot with pore.
17A. No black mark; body speckled yellow and brown.	17B. Black mark below mentum; body otherwise nearly colorless.
18A. Spermatophore with cuticularized distal crook and barb.	18B. Spermatophore not cuticularized; no crook or barb.
19A. Egg diameter *ca.* 87 μm.	19B. Egg diameter *ca.* 55-60 μm.

Evidence that these seven world species are not closely related comes from their protoconchs, some of which are various in morphology. In the eastern American *Fargoa* and *Boonea* species I have studied, protoconch morphology follows generic lines, not larval ecology (Robertson, 1986; also intimated by Graham, 1988). Protoconchs are monomorphic generically, whether or not planktotrophy or lecithotrophy occurs (there may be size differences).

It follows that the larval ecology of pyramidellids cannot be determined from shell characters alone. I now consider the chiton-parasitizing *Odostomia chitonicola* E. A. Smith, 1899, of South Africa very doubtfully a true *Odostomia* and the protoconch far from necessarily indicating lecithotrophy (Robertson and Orr, 1961).

Surprisingly, more pyramidellid generic names may be needed. According to Wenz (1940) there are 130 valid fossil and Recent genera and subgenera, rather few considering that there are reported to be thousands (?) of species world-wide.

The genera *Boonea* and *Fargoa* are better characterized from each other than any other pair of pyramidellid genera, being based on a mixture of shell and animal characters — not only spermatophores (Robertson, 1978). Admittedly, though, the two genera are not yet well distinguished from other pyramidellid genera for which animal characters are so far lacking. Thus *Boonea* and *Fargoa* could be synonyms of earlier named genera that are unstudied biologically. The type species of these other genera might have quite dissimilar shell sculptures, impeding orthodox taxonomy.

This paper indicates strongly that the systematics of pyramidellids must become more biologically oriented.

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LITERATURE CITED

Aartsen, J. J. van. 1987. European Pyramidellidae: III. *Odostomia* and *Ondina. Bollettino Malacologico* 23:1-33.

Abbott, R. T. 1974. *American Seashells; the Marine Mollusca of the Atlantic and Pacific Coasts of North America, 2nd ed.* Van Nostrand Reinhold Co., New York. 663 pp.

Andrews, J. 1971. *Sea Shells of the Texas Coast.* University of Texas Press, Austin. xvii + 298 pp.

Bartsch, P. 1909 (February). Pyramidellidae of New England and the adjacent region. *Proceedings of the Boston Society of Natural History* 34:67-113.

Bartsch, P. 1955. The pyramidellid mollusks of the Pliocene deposits of north St. Petersburg, Florida. *Smithsonian Miscellaneous Collections* 125(2):1-102.

Blaney, D. 1904. List of shell-bearing Mollusca of Frenchman's Bay, Maine. *Proceedings of the Boston Society of Natural History* 32:23-42.

Brocchi, G. 1814. *Conchiologia fossile subapennina....* 2 vols. Milano. 1:56 + lxxx + 240 pp.; 2:241-712.

Bush, K. J. 1909. Notes on the family Pyramidellidae. *American Journal of Science* (4)27:475-484.

Castellanos, Z. A. de. 1982. Los Pyramidellidae de la Republica Argentina (Moll. Entomotaeniata). *Comunicaciones del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia".... Hidrobiologia* 2:61-85.

Clench, W. J. and R. D. Turner. 1950. The western Atlantic marine mollusks described by C. B. Adams. *Occasional Papers on Mollusks* [Harvard University] 1:233-403.

Coomans, H. E. 1962. The marine mollusk fauna of the Virginian area as a basis for defining zoogeographical provinces. *Beaufortia* 9:83-104.

Dall, W. H. 1888. Some American conchologists. *Proceedings of the Biological Society of Washington* 4:95-134.

Dall, W. H. and P. Bartsch. 1909. A monograph of west American pyramidellid mollusks. *United States National Museum Bulletin* 68:i-xii + 1-258.

Fretter, V., A. Graham, and E. B. Andrews. 1986. The prosobranch molluscs of Britain and Denmark. Part 9 - Pyramidellacea. *Journal of Molluscan Studies* supplement 16:557-649.

Gould, A. A. (ed. W. G. Binney). 1870. *Report on the Invertebrata of Massachusetts.... 2nd edition, comprising the Mollusca.* Wright and Potter, Boston. vii + 524 pp.

Graham, A. 1988. Molluscs: prosobranch and pyramidellid gastropods. *In: Synopses of the British Fauna* (new ser.), no. 2, 2nd ed., D. M. Kermack and R. S. K. Barnes, eds. pp. i-iv, 1-662. E. J. Brill/W. Backhuys, Leiden.

Johnson, C. W. 1915. Fauna of New England. 13. List of the Mollusca. *Occasional Papers of the Boston Society of Natural History* 7:1-231.

Johnson, C. W. 1934. List of marine Mollusca of the Atlantic coast from Labrador to Texas. *Proceedings of the Boston Society of Natural History* 40:1-204.

LaFollette, P. I. 1977. Inbreeding and intraspecific variation in *Chrysallida* Carpenter, 1857 (Gastropoda: Pyramidellidae). *The Western Society of Malacologists Annual Report* 10:18-23.

Laseron, C. F. 1959. The family Pyramidellidae (Mollusca) from northern Australia. *Australian Journal of Marine and Freshwater Research* 10:177-267.

Mayhew, R. and F. Cole. 1995. A taxonomic discussion and update of shell-bearing marine molluscs reported from the NW Atlantic north of Cape Cod (including western Greenland) and the Canadian Arctic. *Of Sea and Shore* 18:99-116.

Merrill, A. S., R. C. Bullock, and D. R. Franz. 1978. Range extention of mollusks from the Middle Atlantic Bight. *The Nautilus* 92:34-40.

Miller, W. III. 1983. Survey of the pyramidellid gastropods in the Wassaw Sound area, coastal Georgia. *The Nautilus* 97:93-98.

Nomura, S. 1937. Additional Pyramidellidae from Siogama Bay, with remarks on the molluscan fauna, especially Pyramidellidae from Sagami Bay; being a comparative study. *Saito Ho-on Kai Museum Research Bulletin* 13:11-107.

Odé, H. 1967. Recent finds at Palacios. *Texas Conchologist* 4:23-24.

Odé, H. 1968. Random notes a propos a beachdrift sample from Matagorda Beach - part II. *Texas Conchologist* 5:17-19.

[Odé, H.] 1969. *Odostomia seminuda* C. B. Adams, 1839. *Texas Conchologist* 6:36, 42.

Odé, H. 1971. *Odostomia dianthophila* Wells and Wells, 1961. *Texas Conchologist* 7:89.

Odé, H. 1993. Distribution and records of the marine Mollusca in the northwest Gulf of Mexico (a continuing monograph). *Texas Conchologist* 30:23-32.

Odé, H. 1994. Distribution and records of the marine Mollusca in the northwest Gulf of Mexico. (A continuing monograph: superfamily Pyramidelloidea, family Pyramidellidae part IV). *Texas Conchologist* 31:9-32.

Odé, H. and A. B. Speers. 1972. Notes concerning Texas beach shells. Superfamily Pyramidellacea. Part II. The odostomiid genera and the genus *Longchaeus*. *Texas Conchologist* 9:1-17.

Orbigny, A. d'. 1842. Mollusques, atlas. *In: Histoire Physique, Politique et Naturelle de l'Ile de Cuba,* by R. de la Sagra. Paris.

Porter, H. J. 1976. Spiral cord variation of *Odostomia impressa* (Say) and *O. seminuda* (C. B. Adams) family Pyramidellidae. *Bulletin of the American Malacological Union* for 1976:38-41.

Porter, H. J., L. A. Howie, and R. B. Deriso. 1979. Morphometric character variation in *Boonea impressa* (Say) and *B. seminuda* (C. B. Adams) - family Pyramidellidae. *Bulletin of the American Malacological Union* for 1979:43-48.

Roberge, A. G. 1968. *Odostomia dianthophila* (Gastropoda, Pyramidellidae) from Buzzard's Bay, Mass., a northern range extension. *The Nautilus* 81(4):iii.

Robertson, R. 1967. The life history of *Odostomia bisuturalis*, and *Odostomia* spermatophores (Gastropoda: Pyramidellidae). *Year Book of The American Philosophical Society* for 1966:368-370.

Robertson, R. 1978. Spermatophores of six eastern North American pyramidellid gastropods and their systematic significance (with the new genus *Boonea*). *Biological Bulletin* 155:360-382.

Robertson, R. 1985. Four characters and the higher category systematics of gastropods. *American Malacological Bulletin* special edition 1:1-22.

Robertson, R. 1986. Pyramidellid larval ecology and systematics. *In: Ninth International Malacological Congress, Abstracts*, D. Heppell, ed. p. 72. Unitas Malacologica, Edinburgh, Scotland.

Robertson, R. and T. Mau-Lastovicka. 1979. The ectoparasitism of *Boonea* and *Fargoa* (Gastropoda: Pyramidellidae). *Biological Bulletin* 157:320-333.

Robertson, R. and V. Orr. 1961. Review of pyramidellid hosts, with notes on an *Odostomia* parasitic on a chiton. *The Nautilus* 74:85-91.

Saurin, E. 1958. Pyramidellidae de Pho-Hai (Sud Viet-Nam). *Annales de la Faculté des Sciences de Saigon* for 1958:63- 86.

Saurin, E. 1959. Pyramidellidae de Nha-Trang (Viet-Nam). *Annales de la Faculté des Sciences de Saigon* for 1959:223-283.

Scheltema, A. H. 1965. Two gastropod hosts of the pyramidellid gastropod *Odostomia bisuturalis. The Nautilus* 79:7-10.

Stimpson, W. 1851a (June). [Several species of testaceous Mollusca new to Massachusetts Bay, including new species.] *Proceedings of the Boston Society of Natural History* 4:12-18.

Stimpson, W. 1851b (December) [On several new species from New England.] *Proceedings of the Boston Society of Natural History* 4:113-114.

Stimpson, W. 1851c (errata dated Nov. 25). *Shells of New England. A Revision of the Synonymy of the Testaceous Mollusks of New England, with Notes on their Structure, and their Geographical and Bathymetrical Distribution.* Phillips, Samson and Co., Boston. 58 pp.

Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Meddelelser fra Kommissionen for Danmarks Fiskeri- og Havundersøgelser,* ser. *Plankton* 4(1):1-523.

Tryon, G. W., Jr. 1873. *American Marine Conchology*. G. W. Tyron, Philadelphia. vi + 208 pp.

Wells, H. W. and M. J. Wells. 1961. Three species of *Odostomia* from North Carolina, with description of new species. *The Nautilus* 74:149-157.

Wells, H. W. and M. J. Wells. 1969. New host and distribution records of *Odostomia dianthophila. The Nautilus* 82:109-110.

Wenz, W. 1940. Gastropoda. *In: Handbuch der Palaozoologie,* O. H. Schindewolf, ed. 6, Teil 4, Lief. 6:721-960. Borntraeger, Berlin.

Winkley, H. W. 1901. Variations in *Odostomia. The Nautilus* 14:126-127.

Winkley, H. W. 1909 (July). New England Pyramidellidae, with description of a new species. *The Nautilus* 23:39-40.

Winkley, H. W. 1912. New England notes. *The Nautilus* 26:54-55.

Winkley, H. W. 1916. Collecting at Nantucket and Martha's Vineyard. *The Nautilus* 29:109-110.

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Mörch's worm-snail taxa (Caenogastropoda: Vermetidae, Siliquariidae, Turritellidae)

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Abstract: Otto Mörch's (1828-1878) marine caenogastropod "worm-snail" taxa are reviewed based upon research on his published descriptions and original material deposited in museum collections in Copenhagen, Berlin, and London. Mörch's publications introduced more than 220 new names for Vermetidae *sensu lato* (now members of Vermetidae, Siliquariidae, and Turritellidae), ranging from regular genus- and species-group names to "formae" and "varieties" of unsettled status. Previous claims that Mörch's complex taxonomic treatment might not be binominal are refuted. Nine genus-group names and 50 species-group names were introduced by Mörch for worm-snails and each is discussed. Over 160 "varieties" and "forms" were not introduced as taxonomic names but as morphological descriptions and are rejected as having no nomenclatural status. *Siliquaria lactea* Lamarck, 1818, is designated as type species for *Pyxipoma* Mörch, 1861 (Siliquariidae).

Key words: Gastropoda, Mollusca, nomenclature, taxonomy

> "The literature of *Vermetidae* is in a most confused state at present, the labors of Mörch being as remarkable for their obscurity as for their extent, and that is considerable." - H. A. Pilsbry (1892:472)
>
> "The present posture of binomial nomenclature is well illustrated in [Mörch's] most elaborate paper, which few naturalists have professed to understand." - P. P. Carpenter (1864:558)

Marine "worm-snails" (here meant to comprise the polyphyletic assemblage of caenogastropod Vermetidae, Siliquariidae, and *Vermicularia* of Turritellidae) are a notoriously problematic group in gastropod systematics. Most species have their postlarval shells firmly cemented to hard substrata or embedded in sponges and thus are difficult to collect and poorly represented in existing collections. Moreover, their irregular "uncoiled" shell growth, often habitat-influenced shell morphology, and highly derived anatomical features do not readily allow comparison with normally coiled, free-living gastropods. In fact, their shells are frequently confused with superficially similar calcareous structures such as polychaete tubes, linings of bivalve burrows, scaphopod fragments, and even fossilized vertebrate bones (Boettger, 1963; ten Hove and van den Hurk, 1993; ten Hove, 1994).

One of the few workers who ever specialized in this group was the Danish malacologist Otto Andreas Lowson Mörch (1828-1878), also spelled Mørch or Moerch, curator at the Zoologisk Museum of the University of Copenhagen. Between 1848 and 1877, he published over 100 scientific contributions in Danish, Latin, French, German, and English (see bibliographies by Collin, 1878, and Schlesch, 1943). Among his better-known works are faunistic studies on mollusks from boreal regions (*e.g.* Denmark, Faroe Islands, Greenland, Iceland, and Spitsbergen), Central America, and the West Indies. His type material of western Central American mollusks was studied by Keen (1966). Eleven of Mörch's works dealt exclusively or extensively with the systematics of "worm-snails" (Vermetidae in the broad sense at the time), and he also published a monograph on the tube-building serpulid worms (Mörch, 1863).

His series of worm-snail papers (Mörch, 1859, 1860a, b, c, 1861a, b, 1862a, b, 1865, 1871, 1877) introduced over 220 new names at levels ranging from genus to "variety." Mörch's descriptions were usually short and rarely illustrated. His worm-snail information was gathered exclusively from existing publications and collections rather than from original field work [which was restricted to an area without living worm-snails, a few localities in Denmark (Schlesch, 1943)]. The main collections used were those in Copenhagen where his duties included integrating the holdings of the Royal Natural History Museum ("Mus. Reg.," "Royal Mus.") with those of the University ("Coll. Univ."), as well as the collections in London [British Museum (Natural History), mainly coll. Cuming] and Berlin (Zoologisches Museum, coll. Dunker). Depending on second-hand information such as the notoriously poor label data in the Cuming collection, the locality data for his taxa were often vague or erroneous, a fact of which Mörch himself was well aware. The type locality of *Thylacodes oryzata* Mörch, 1862, for instance, was given as "China" (based on Cuming's data), although Mörch and subsequent authors assumed a more likely origin from western Mexico.

The main reason, however, why later authors have had difficulties in following and adopting Mörch's taxonomic decisions and resulting classification was his elaborate system of introducing names for variations and growth stage morphs. Carpenter (1864:558) chose an extreme example when he expressed his bewilderment at Mörch's "Review of the Vermetidae" (1861-1862): "The shell described [by Carpenter, 1857b] is quoted as "*Vermetus (Thylacodus) contortus,* var. τ. *contortula (Thylacodus),* forma 1, *Thylacodus* (?) *contortus,* var. *indentata*, Cpr." Mörch (1865:98) tried to explain that "I have never intended to introduce a tri- or poly-nomial nomenclature; but I believe it is necessary to name the different varieties, forms, and deviations, as well as the differences of sex and age." Nevertheless, later workers found the study of Mörch's system "even more perplexing than that of the specimens themselves" (Tryon, 1886:164). Iredale (1937:254) stated that Mörch's "conclusions are not easy to follow; apparently they were not regarded as final, but rather preliminary," then proceeded with the introduction of his own new genus- and species-group names. Like Iredale, most other 20th-century authors have ignored Mörch's taxonomic maze except for a few easily recognizable species such as the Hawaiian vermetid *Dendropoma platypus* (Mörch, 1861) and the Australian siliquariid *Tenagodus ponderosus* (Mörch, 1860).

With over 200 species-level, "form," and "varietal" names of undetermined status in Mörch's vermetid papers, his monographs represent an obstacle for future work. This paper clarifies the taxonomic status of Mörch's names introduced for Vermetidae *sensu lato*. Based upon critical evaluation of the literature and study of Mörch's original material and labels in the collections in Copenhagen (Zoologisk Museum, University of Copenhagen, ZMUC), London (The Natural History Museum, BMNH) and Berlin (Zoologisches Museum, Naturkunde-Museum der Humboldt-Universität, ZMB), it is herein shown that his system is less enigmatic than it might have seemed to earlier workers. This new interpretation results in a drastic reduction of "potentially available but currently unused" taxonomic names and thus contributes toward taxonomic stability in the three families of "worm-snails."

INTERPRETATION OF MÖRCH'S FORMAT

Mörch's worm-snail works contain entries as in the following format[1]:

Vermetus (Thylacodus) albus, Smith, 1799, (*Aletes*), Forma 1. *electrina var.* αα, *perlata, linea castanea,* juv.

While this arrangement appears to contain three genus-group and at least two species-group names, it does not. It is just an unusual approach combining a standard taxonomic name, "*Vermetus (Thylacodus) albus* Smith, 1799," with description of the morphological condition of the individual specimen(s) at hand. Mörch usually worked with only a few specimens of each species and was determined to describe the full range of diversity encountered.

The above entry can be interpreted by separating the taxonomic and morphological parts as in Table 1. Only genus, subgenus, author, and date form the taxonomic component. All other information represents a morphological description and comparison. While some parts of this interpretation were taken from Mörch's (1865) statements as quoted above, there is additional direct evidence in his original articles. Despite the spread of publications over 18 years and several journals and languages, his treatment remained so consistent that the papers can be evaluated together. Mörch was careful and consistent in applying priority rules to his taxonomic decisions whenever dealing with formal names at the genus- and species-group levels. With two exceptions that could have been editorial lapses (*Siphonium adamsii* and *Vermetus balanitintinnabuli*, see below), new taxa were clearly marked by "n. sp.," "Mörch," "nobis," or "n." This is in stark contrast to the treatment of "varieties" in these same papers. These names were never

Table 1. Interpretation of taxonomic and morphological components of Mörch's descriptive format based on example given in text.

TAXONOMIC PART	
Vermetus	Genus
(*Thylacodus*)	Subgenus
albus	Species
Smith, 1799	Original author and date
MORPHOLOGICAL PART	
(*Aletes*)	Genus-group name used to indicate overall morphology or growth stage of particular specimens (in the sense of "looks like...") [sometimes placed after name of "variety"]
Forma 1	grouping of several forms (species or "varieties") with similar morphology [rarely used]
electrina	Latin name of that "Forma" [rarely used]
var. αα	sequential Greek lettering for morphological "varieties" in each species
perlata	Latin name or description for that "variety"
linea castanea	additional words of Latin description [rarely used]
juv.	ontogenetic stage of specimen [rarely used]

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[1] To demonstrate all encountered categories in a single example, I present a composite example that does not itself occur in Mörch's publications.

cited with an author's name or indicated as being "new," and Mörch often used a descriptive word for his varietal names rather than the available "senior synonym" among the literature references listed for that variety (*e.g.* "cinerea" describing the variety also including the available name *Vermetus radicula* Stimpson, 1851). Not all of his varietal descriptions consisted of single words, and Mörch never went back to one of these descriptive terms when he subsequently decided to elevate one of these "morphs" to species rank. He also made repetitive use of the same name (*e.g.* "repens") to indicate that several species occurred with similar morphology.

Subsequent authors (Tryon, 1886; Clessin, 1901-1912; Ruhoff, 1980) only copied or paraphrased the "varieties" in listings, without validating any of the names. Some of the varietal names were used in binominal fashion in figure captions by Mörch (1861b), Tryon (1886), and Clessin (1912). However, it can be demonstrated that these two-word citations were used for brevity, not as taxonomic statements, as each accompanying main text clearly gives the names with their "varietal" rank. Only three cases were found in which such names were validated by a later author. The first is Mörch's *Vermetus (Petaloconchus) intortus* Lamarck var. τ woodi (1862a:354), which was used as *"Vermetus (Petaloconchus) intortus* var. *woodii* Mörch" by Sacco (1896:9). The second is Mörch's *Vermetus (Vermetus) renisectus* (Carp.) Mörch Var. β gordialis (*Thylacodus*) (1862a:346-347), which was used as "*Vermetus renisectus gordialis* Mörch" by Iwakawa (1909:79). The third name is Mörch's *Vermetus (Vermetus) conicus* Dillwyn and Wood var. ζ retifera (Aletes) (1862a:343), which Turton (1932:127) used as *"Vermetus conicus retifera,* Mörch, 1861." These names are here interpreted as subspecific names (ICZN, 1985:Art. 45 [ii]) introduced by Sacco (1896), Iwakawa (1909), and Turton (1932), respectively.

An additional "category," only occasionally used by Mörch, was "Forma." He used this to organize several species or varieties into groups of similar morphology. Again, these names were employed in the sense of "this specimen looks like ...," as demonstrated by his statement (1862a:336): "I have seen a specimen of the last species which is in the fore part an *Aletes*, and in the first whorls a plaited *Vermetus*." Names used for his "Forma" groups were (a) previously introduced generic names (*e.g. Thylacodus* Mörch, 1860b, used as Forma in 1862a:336) or, (b) varietal names previously introduced by Carpenter (*e.g.* *"woodwardi*, Carp."; based on *"Petaloconchus? renisectus* var. *woodwardii"* Carpenter, 1857a:313), or (c) Latin adjectives employed in the same fashion as Mörch's varietal names. There are only two occurrences of this last category, a *"Bivonia quoyi,* H. & A. Adams. Forma 1. aspersa" (1862b:60) and *"Vermetus varians*, D'Orb. Forma 1. electrina" (1862a:340). The latter name was used again, this time in a formal manner, when Mörch introduced the new species *Vermetus electrinus* in 1877.

In summary, from the foregoing analysis and the following taxonomic sections:

(1) Mörch's worm-snail taxonomy is complicated but can be accepted as binominal.

(2) Nine genus-group names were first introduced in these papers; three additional ones are shown to have been credited to Mörch erroneously.

(3) 50 nominal species were first introduced in these papers.

(4) Over 160 named "varieties" were included, but not introduced as formal taxonomic names. They were clearly infrasubspecific in their original forms and can be rejected as having no nomenclatural status (only three validations of such varietal names by subsequent authors have been found).

(5) Interpretation of Mörch's species should be restricted to the material and description of the nominate form alone. Much of this "varietal" material is not conspecific with the nominate form (pers. obs.). For taxonomic purposes it should be noted that the varietal material does not qualify as part of a syntypic series, because ICZN (1985) Article 72(b)(i) specifically excludes specimens from the type series of a nominal species-group taxon if the original author referred to them as distinct variants "*e.g.*, by name, letter, or number."

(6) The two names newly introduced as "Forma" were not presented as proper taxonomic names and can be rejected as having no nomenclatural status.

TAXONOMIC SECTION

This is an annotated list of genus- and species-group names introduced by Mörch for "Vermetidae" *sensu lato*. Current taxonomic placement is noted in square brackets at the end of each paragraph. Names are presented as given by Mörch, with adjustments as mandated by ICZN (1985) Article 32(d), "Correction of incorrect original spellings."

GENUS-GROUP NAMES

"*Bivonia* 'Mörch'." Iredale (1937:254) referred to a "*Bivonia* Mörch, 1862," concluding that it was "questionable whether this is the same as Gray's *Bivonia.*" The name *Bivonia* Gray has been the focus of extensive discussion in the literature (*e.g.* Monterosato, 1892:15; Sacco, 1896:13). *Bivonia* Gray was first introduced by Gray (1842:90). This could have been dismissed as a *nomen nudum* if Gray (p. 62) had not added a description of the operculum of the "Bivinae" (apparently meant to include the species of *Bivonia*). A type species, *Vermetus glomeratus* Bivona-Bernardi, 1832, was subsequently selected by Gray (1847:156). Gray (1850:82) then altered the diagnosis of the operculum, apparently copying from Philippi's (1836:170) description of *Vermetus triquetrus* Bivona-Bernardi, 1832. This in turn seems to have influenced Mörch (1862b:54) to accept *Bivonia* Gray "1850" based on *Vermetus triquetrus* (he previously stated that *Bivonia* Gray, "1847" was founded for *Vermetus glomeratus* [1859:360]). The genus-group name *Bivonia* Gray cannot be used as a valid name because it is preoccupied by *Bivonia* Cocco, 1832. [Crustacea]

Burtinella Mörch, 1861b:147; pro *Moerchia* Mayer-Eymar, 1860 [July or August], (non *Moerchia* [as *Mörchia*] A. Adams, 1860 [April]; nec *Moerchia* [as *Mörchia*] Martens, 1860 [? November]). The description of *Moerchia* Mayer-Eymar was founded on *Solarium nysti[i]* Galeotti, 1837 (see also Gardner, 1939:17; Schmidt, 1955:164), with *Serpula turbinata* Philippi, 1847, also included as a possible synonym. Indicated as belonging to the serpulid polychaete genus *Hydroides* Gunnerus, 1768, by Mörch (1863:468-469), but treated as vermetid gastropod by subsequent authors (Tryon, 1886:167; Sacco, 1904:127; Cossmann, 1912:140; and Wenz, 1939 in 1938-1944:677, the last three erroneously citing *Serpula turbinata* Philippi "1846" as type species). Schmidt (1955:164) also accepted *Burtinella* as a valid genus of Vermetidae [*sensu lato*], based on the additional species included by Mörch (1861b). However, *Burtinella* is here interpreted as a new replacement name (*nomen novum*; ICZN, 1985:Art. 12[b][3]) for *Moerchia* Mayer-Eymar, and thus shares the same type species (ICZN, 1985:Art. 67[h]). This type species, *Solarium nystii*, was recognized by Schmidt (1955:176) as a species of the fossil serpulid worm genus *Rotularia* Defrance, 1827. *Burtinella* Mörch is here placed in synonymy of *Rotularia*. [Polychaeta: Serpulidae]

Dendropoma Mörch, 1861b:153 (as a section of *Siphonium*); type species by subsequent designation (Keen, 1961:189): *Siphonium (D.) lituella* Mörch, 1861. Placed on the Official List of Generic Names in Zoology (ICZN, 1987:57). [Vermetidae]

Dofania Mörch, 1860a:34 (as a subgenus of *Vermetus*) = "Vermiculus" Costa, 1776, non Lister, 1688 (the latter was introduced as *Vermiculus* Mörch, 1860, see below); type species by subsequent designation (Bucquoy *et al.*, 1884:238): "Vermetus dofan Adanson = Vermetus Goreensis Gmelin" = *Serpula goreensis* Gmelin, 1791. Keen (1961:189) stated: "Adanson's type specimen of Le Dofan seems no longer to be discoverable, according to Fischer-Piette (1942:264). As the type figure does not permit of specific determination, the generic name is at present unusable." [Vermetidae]

Pyxipoma Mörch, 1861a:409 (as subgenus of *Tenagodus*); including four nominal species (*Siliquaria lactea* Lamarck and *tahitensis, anguillae, cylindrella* of Mörch). Several subsequent authors referred to an Eocene fossil as type species of this taxon, *e.g.* Cossmann (1888:317 [321], citing "*T. multistriatus,* Desh." and 1912:149, citing "*Siliquaria multistriata* Desh."); and Wenz (1939 in 1938-1944:681, citing "Typus: *T. (P.) multistriatus* (Deshayes) [*Siliquaria*]"). However, this nominal species cannot be fixed as type species because it was not originally included when *Pyxipoma* was introduced (ICZN, 1985:Art. 67[g]). *Siliquaria lactea* Lamarck, 1818, is here selected as type species by subsequent designation. [Siliquariidae]

"*Siphonium* 'Browne' Mörch." Fischer (1885:691), Tryon (1886:167), Sacco (1904:127), and Cossmann (1912:134) credited this name to Mörch. Keen (1961:198) listed "*Siphonium* Mörch, 1859 [not Link 1807]" in the synonymy of *Dendropoma* Mörch, 1859. Mörch referred to "Siphonium Browne," giving the date as either "1757" (1859:348) or "1756" (1859:353). This pre-Linnean name had been used in binominal fashion before Mörch (1859) by J. E. Gray, first tentatively in synonymy of *Vermetus* (1847:156), and later (1850:82) as a genus for several Indo-Pacific species. Mörch subsequently (*e.g.* 1861b:152; 1871:128) credited the name to Gray. Keen (1980) discussed other occurences of 'Siphonium' in a later, but still non-binominal edition of Browne's work (1789), as well as in the invalid work by Gronovius (1781) where it was only cited in synonymy. *Siphonium* J. E. Gray, 1847, cannot be used as a valid name because it is a junior homonym of *Siphonium* Link (1807:9; Cephalopoda). [Vermetidae]

Stephopoma Mörch, 1860a:42 (as genus); two nominal species included (*Vermetus roseum* Quoy and Gaimard and *S. pennatum* Mörch). Type species by subsequent designation (Cossmann, 1912:134): *Vermetus roseus* Quoy and Gaimard, 1834. Subsequently employed as subgenus of *Vermetus* by Mörch (1871:128). This is "*Stephostoma*" as used by

Clessin (1903:90-92). [Siliquariidae]

Tetranemia Mörch, 1859:353 (as subgenus of *Serpulus*); type species by monotypy: *Serpulus dentiferus* [error for *dentifer*] "Lamarck" *sensu* Quoy and Gaimard, 1834, non *Serpula dentifera* Lamarck, 1818 [subsequently named as *Thylacodes longifilis* Mörch, 1862; see below]. Later treated as a subgenus of *Thylacodes* by Mörch (1862b:79). This is "*Tetranema* Mörch, 1859" of Cossmann (1912:138) and "*Tetraneusia* Mörch, 1859" of Wenz (1939 in 1938-1944:677). [Vermetidae]

"*Thylacodes* 'Guettard' Mörch." Mörch (1862b:64) referred to "*Thylacodes* Guettard, 1774" [error for 1770]. Keen (1961:191), who dismissed Guettard's (1770) work as non-binominal, credited the name to Mörch (1862b) and, by subsequent designation, fixed the type species as "*Serpulorbis polyphragma* Sassi [error for Sasso], 1827 = *Serpula arenaria* Linné, 1758." This would have made "*Thylacodes* Mörch" a junior objective synonym of *Serpulorbis* Sasso, 1827. However, genus-group names introduced in Guettard's (1770) work are available (for discussion involving other such names, including *Tenagodus, Kuphus, Uperotus,* and *Brechites*, see Bieler, 1992). Guettard's original spelling of the name was *Tulaxodus* (1770:143). *Tulaxodus* Guettard, 1770, is an unused senior synonym in the sense of ICZN (1985) Article 79(c), and should be suppressed for the sake of nomenclatural stability. The spelling *Thylacodes* is an intentional emendation (unjustified in the sense of ICZN, 1985:Art. 33[b]), and that name is therefore available with its own author and date. The first to introduce it was not Mörch (1862b), but Agassiz (1848:1067). Keen's type designation is here accepted for *Thylacodes* Agassiz, 1848, making it a junior objective synonym of *Serpulorbis* Sasso, 1827. *Thylacodus* 'Guettard' Marschall, 1873, is another unjustified emendation of *Tulaxodus*; the name is preoccupied by *Thylacodus* Mörch, 1860 (see below). [Vermetidae]

Thylacodus Mörch, 1860b:78 (as subgenus of *Vermetus*). Mörch (1860b:77-78) included two species in his new taxon, *"Bivonia subcancellata"* [*Vermetus subcancellatus* Bivona-Bernardi, 1832] and "*Vermetus (Thylacodus) contortus (Bivonia)* Carpent." [*Bivonia contorta* Carpenter, 1857b:305], but stated that he described the new subgenus "für *Bivonia subcancellata* Biv." While this could be considered an original type species designation in the sense of ICZN (1985) Article 67(c), Keen (1961:191) selected the other species, *Bivonia contorta* Carpenter, as type species by subsequent designation. Keen's designation is here accepted for the sake of nomenclatural stability, resulting in *Thylacodus* Mörch, 1860b, being a junior objective synonym of *Thylaeodus* Mörch, 1860a (see below). *Thylacodus* Mörch, 1860, predates *Thylacodus* Marschall, 1873, an unjustified emendation of *Thylaxodus* Guettard, 1770. [Vermetidae]

Thylaeodus Mörch, 1860a:48; type species by subsequent designation (Keen, 1961:191): *Bivonia contorta* Carpenter, 1857b. [Vermetidae]

Vermiculus Mörch, 1860a:27; ex Lister, 1688 (pre-Linnean), (non Dalyell, 1853); with numerous species originally included. No type species designation seems to exist. *Vermicularia* Lamarck appeared in Mörch's (1860a) synonymy for *Vermiculus* "Lister," and Mörch later (1877:110) placed "*Vermiculus* Lister, Mörch" in synonymy of *Vermicularia. Vermiculus* Mörch, 1860, is a junior homonym of *Vermiculus* Dalyell, 1853, and was placed on the Official Index (ICZN, 1958:Direction 102). [Turritellidae]

A Note on *Vermicularia* Lamarck, 1799:

Lamarck (1799:78) introduced this genus, with "*Serpula lumbricalis.* Lin." as type species by monotypy. This is *Serpula lumbricalis* Linné, 1758, originally introduced (1758:787) with references to four pre-Linnean illustrations, all showing shells of the turritellid genus *Vermicularia* in today's sense. The type locality is given as "Habitat in Indiis," which is in error because the genus *Vermicularia* does not appear to have extant species in that region.

Daudin (1800:34) introduced the genus *Vermetus*, with the type species (by tautonomy, based on "Le Vermet" of Adanson, 1757:160) *V. adansonii* Daudin, 1800 (see exhaustive discussion by Keen, 1961:186-188). Daudin erroneously listed Linné's *Serpula lumbricalis* in synonymy of *"Le Vermet."* Several subsequent authors thus confused *Vermetus* "Adanson" Daudin (the name-bearing genus of Vermetidae), with *Vermicularia* Lamarck (a genus of Turritellidae).

Lamarck (1801:97) gave a more extensive description of *Vermicularia,* again citing Linné's species (as "*Vermicularia lumbricalis.* n[obis]. *Serpula lumbricalis.* Lin.") and adding information based on Adanson (1757) from Senegal specimens. Lamarck later (1822:225) referred to the Senegal material, this time using the name *Vermetus lumbricalis* without indicating original authorship.

Subsequent authors (*e.g.* Deshayes, 1843:66) argued for the existence of two species named *lumbricalis*, of Linné and Lamarck, respectively. Fischer-Piette (1942:260, pl. 9, figs. 3-5) described the original material of *Vermetus adansonii* in the Paris Museum (see also Keen, 1961:193, figs. 4-7). Mermod and Binder (1963:159) then described *Vermicularia* specimens in Lamarck's collection in Geneva (3 shells, *vidi*) and cited them as type material of "*Vermetus lumbricalis* Lamarck, 1822." The latter is here viewed as a misinterpretation because Lamarck had clearly referred to Linné's species.

SPECIES-GROUP NAMES

adamsii, Siphonium?, Mörch, 1859:359; "Bornéo (Adams.)," based on "*V*[*ermetus*]?? ----, n. s. Adams, MSS" in J. E. Gray (1850:82; M. E. Gray, 1850:pl. 82, fig. 1, "Mr. A. Adams's drawing"). Type locality corrected to "From Japan, according to Mr. A. Adams; but not from Borneo" by Mörch (1865:99). Subsequently cited as *Thylacodes adamsii* (Mörch, 1859) by Mörch (1865:99). Adams (1864:141) described the coloration and features of the living animals from Japan under the name of "*Serpulus Adamsi*, Mörch," and noted that the "same species has recently been decribed by Dr. Dunker, in his 'Mollusca Japonica,' as *Serpulorbis imbricatus*. Mörch (1865:99) recognized *Vermetus imbricatus* Dunker, 1860 (:240; 1861:17, pl. 2, fig. 18; Recent, Japan), as preoccupied by *V. imbricatus* Sandberger, 1859 (1859:222, pl. 12, fig. 4; Tertiary, Germany), and stated that "*Thylacodes imbricatus*, Dkr ... must therefore be named *Thylacodes adamsii*, Mörch." *Siphonium adamsii* was not introduced as a replacement name, its synonymy with *Vermetus imbricatus* Dunker is subject to interpretation. The synonymy was accepted by some workers (*e.g.* Clessin [1912:117] who gave preference to *T. adamsii*, and Kuroda and Kinoshita [1951:12] who gave preference to *V. imbricatus*), but preoccupied "*imbricatus* Dunker" nevertheless became a widely used name. Keen (1973:5), who apparently overlooked Mörch's 1859 introduction of the name, credited the name to Adams (as "*Serpulus adamsi* A. Adams, 1864, ex Mörch MS") and erroneously dismissed it as an "unneeded new name" for *Vermetus imbricatus* Dunker. The lectotype of Dunker's nominal species is in Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt (SMF 314255; see Janssen, 1993:411); material of *S. adamsii* was not found in BMNH (K. Way, pers. com., 1995). [Vermetidae]

anellum, Vermetus (*Strebloceras*??), Mörch, 1862a:359; "California, on *Haliotis tuberculatus* (Reeve), with *Siphonium megamastus* [sic] (Mörch)"; no collection indicated. Carpenter (1864:557) noted: "Not a Californian *Haliotis*. The diagnosis, however, exactly accords with a Californian shell, which is perhaps the young of *S. squamigerus*. It has no resemblance to *Strebloceras*, Cpr., P.Z.S. 1858, p. 440, which is a genuine Caecid." Part of this was rejected by Mörch (1865:99) who responded: "As this species is always sinistral, it cannot be the young of *Vermetus squamigerus*, but is more likely to be a *Spirorbis* [Polychaeta]." Tryon (1886:173, pl. 49, fig. 34) described and illustrated a 2-3.5 mm small, strongly axially ribbed, sinistral shell. Oldroyd (1927:51 [= 653]) accepted the taxon as member of the vermetid genus *Petaloconchus*. Material was not found in BMNH (K. Way, pers. com., 1995). [Polychaeta?]

anguillae, Tenagodus (Pyxipoma), Mörch, 1861a:410; "Oc. Atlant. ad ins. Anguillam Antillarum (Dr. Hornbeck)"; 2 syntypes, ZMUC GAS-210 and GAS-211. [Siliquariidae]

balanitintinnabuli [as "balani-tintinnabuli"], *Vermetus (Thylacodus)*, Mörch, 1862a:359; no locality given; "on a valve of *Balanus tintinnabulum* (Mus. Reg.)"; with one named variety from "Ins. Philippin."; 2 syntypes, ZMUC GAS-207, without locality data, from collections of Spengler and King Christian VIII, respectively. [Vermetidae]

bernardii, Tenagodus (Siliquarius), Mörch, 1860c:368; "Hab....? Aggloméré dans une éponge." Tryon (1886:190) subsequently cited "Australia" as the locality, while Clessin (1901:2) gave "Senegal." Holotype, ZMUC GAS-208, coll. Mörch (label with specimen says "Australia?" in Mörch's handwriting). This is the species listed as "*Tenagodus*, sp. n. (coll. Bernardi)" by Mörch (1861a:411) and as "*Siliquaria Bernhardi*" by Sowerby (1878:sp. 9; 1887:165) and Clessin (1901:2). *Tenagodus bernardi* was made the type species of *Hemitenagodus* Rovereto, 1899 (= *Montfortia* Della Campana, 1890, non Récluz, 1843); see Bieler (1992:17). [Siliquariidae]

"*bispinosum* [as *bispinosa*], *Stephopoma*"; cited as of "Mörch Journ. de Conchyl. 1859" by Mörch (1860b:78), but not located in that journal volume; introduced without description and giving locality as "Realejo auf *Crucibulum scutellatum* und *Callopoma fluctuosus* Wood." This form was later treated by Mörch (1861b:151) as a variety of *pennatum*, see below. "*Stephopoma bispinosum* Mörch" is here considered unavailable because its initial introduction

(1860b) did not fulfill the requirements of availability (ICZN, 1985:Art. 12). Its 1861 introduction was as a junior synonym of another species (ICZN, 1985:Art. 11[e]) with subsequent treatment as an infrasubspecific "variety."

constrictor, Bivonia, Mörch, 1862b:63; "Australia (Mus. Cuming)." Potential holotype specimen (BMNH 197877) illustrated as "*Bivona* [sic] *constrictor*" by Hedley (1913:294, pl. 18, fig. 71); Keen (1961:202) cited this as "?Holotype: B.M.(N.H.) Reg. No. 195919." [Vermetidae]

contrarius, Spiroglyphus, Mörch, 1860a:45; "Indes-Orientales, sur le *Polydonta granularis* Bolt."; based on *"Serpula spirorbis"* varieties of several authors. Subsequently merged into variety "immersa" of *Spiroglyphus spiruliformis* De Serres, 1855, by Mörch (1862a:329). [Vermetidae]

cumingii, Tenagodus (Siliquarius), Mörch, 1861a:403; "Ins. Philippin. (coll. Cumingii). Specimen unicum" (p. 404). Philippine specimens ex Cuming, BMNH 1995097 and ZMUC GAS-212; including six named varieties, with material in part present in BMNH. See Tryon (1886:pl. 57, fig. 19, pl. 58, fig. 21; based on Sowerby, 1878:figs. 2, 2b [2b erroneously labelled "3"]) and Sowerby (1887:pl. 1 [480], fig. 4). [Siliquariidae]

cylindrella, Tenagodus (Pyxipoma), Mörch, 1861a:410; "Caput Bonae Spei? [Cape of Good Hope]"; coll. Mörch. One specimen, ZMUC GAS-214 (potential holotype; without locality data but labelled "orig.") fits the stated dimensions when measured as if stretched out. [Siliquariidae]

dacostae [as "Da Costae"], *Spiroglyphus,* Mörch, 1860a:46; based on the specimen illustrated by Costa (1776:pl. "IX" [error for XI] fig. 15; copied in Tryon, 1886:pl. 55, fig. 91); "Indes-Orientales." Holotype, BMNH 195925, *teste* Keen, 1961:206. Subsequently listed as *Siphonium (Stoa) da-costae* by Mörch (1861b:157). [Vermetidae]

dimorphus, Vermiculus, Mörch, 1861b:176; "Ins. Philippin. (H. Cuming)" (p. 177); with one named variety. Holotype, BMNH 1995098, Philippine locality doubtful. See Costa (1776:pl. 10, fig. 6, copied in Tryon, 1886:pl. 56, fig. 2). [Turritellidae: *Vermicularia*]

electrinus, Vermetus, Mörch, 1877:118; "St. Thomas (Riise, Krebs)"; with five named varieties. Raised to species rank from previously mentioned "*Vermetus varians* d'Orbigny Forma 1 electrina" (Mörch, 1862a:340); 15 syntypes, ZMUC GAS-235. [Vermetidae]

encausticus, Tenagodus (Siliquarius), Mörch, 1861a:408; "Ins. Ceylon (E. L. Layard). Specimen unicum in coll. Cumingii." Holotype, BMNH 1995099; figured by Sowerby (1878:pl. 4, fig. 10; 1887:pl. 2 [481], fig. 18). [Siliquariidae]

eruciformis, Thylacodes, Mörch, 1862b:70; "California, on *Crucibulum* ? *umbrella,* Desh., var. (Mus. Cuming)." Holotype, BMNH 195915, illustrated by Keen (1961:203, pl. 54, fig. 3); introduced with two named varieties (material in BMNH 198326 and 198327). Keen (1971:406) placed this in genus *Serpulorbis* and later (1983:10) gave the range as "Head of the Gulf of California to Acapulco." This is "*Thylacodes cruciformis* Mörch" of Carpenter (1864:557). [Vermetidae]

gaederopi, Siphonium, Mörch, 1861b:163; "On *Spondylus gaederopus*: probably from Spain" (1861b:164); several possible syntype lots, ZMUC GAS-215 through GAS-218. [Vermetidae]

"*incisus, Tenagodus (Siliquarius),* 'Chemnitz, 1786' Mörch"; credited to Mörch by Sowerby (1878:sp. 8) and Tryon (1886:188, 238). Mörch (1861a:408) referred to "Helix incisa Chemnitz," a name first made available as *Helix incisa* Gmelin, 1791 (:3630). [Siliquariidae]

leucozonias, Siphonium (Dendropoma), Mörch, 1861b:155; "West Africa"; with one named variety. Lectotype (Keen, 1961:206) and paralectotypes, BMNH 195926/1-2. [Vermetidae]

lilacinus, Vermetus, Mörch, 1862a:352; "Zanzibar (Coll. Dunkeri)"; type lot ZMB unnumbered, *vidi,* with columellar folds typical of *Petaloconchus.* Keen (1961:204) mentioned that "specimens of the 'var. alpha' of Mörch, from Madagascar, are in the B.M.(N.H.) coll., Reg. No. 195922." However, Mörch (1862a:352) described his "Var. α" from "Zanzibar (Mus. Cuming)." [Vermetidae]

lituella, Siphonium (Dendropoma), Mörch, 1861b:154; "California: about ten specimens deeply embedded in the surface of a young *Haliotis splendens* (coll. Cuming)" (p. 155). Lectotype, BMNH 195917, selected and illustrated by Keen (1961:206, pl. 55, fig. 1, upper of two specimens); the same figure is reproduced upside-down in Keen (1971:407, fig. 505), her reference "BM, Lectotype" (p. 947) must accordingly refer to the lower of two animals. Keen (1983:10) described the range as "Southern California to La Paz, Baja California." Placed on the Official List of Specific Names in Zoology (ICZN, 1987:57). [Vermetidae]

longifilis, Thylacodes (Tetranemia), Mörch, 1862b:79; introduced for *Vermetus dentiferus* [error for *dentifer*] "Lamarck" Quoy and Gaimard, 1834 (:291, pl. 67, figs. 27, 28), non *Serpula dentifera* Lamarck (1818:367); "Baie des Chiens-Marins à la terre d'Endracht, sur une Avicule" *teste* Quoy and Gaimard (1834:292). See *Tetranemia* Mörch, 1859. [Vermetidae]

luridum, Siphonium, Mörch, 1861b:164; "Society Islands (coll. Cuming)"; holotype, BMNH 1995091. [Vermetidae]

lyngbyanus, Vermetus (Stephopoma), Mörch, 1871:128; "Kattegat Nordseite der Insel Seeland, auf der Eikapsel von *Raja clavata*"; previously listed as "*Stephopoma* n. sp." by Mörch, 1862b:83. Royal Collection. Based on a brown operculum "very like those of *Stephopoma senticosum*" (1862b:83). Given locality, Denmark, on egg capsule of a ray, highly unlikely for a siliquariid or vermetid. Not located in ZMUC collection (T. Schiøtte, pers. com., 1995). [*Nomen dubium*]

megamastum, Siphonium (Dendropoma), Mörch, 1861b:153, pl. 25, figs. 12, 13; "California?" (p. 154); no collection indicated; with one named variety. Type material could not be located in BMNH as early as 1958 (Keen, 1961:206); however, there is a group of syntypes from California (ex London, 1835), ZMUC GAS-219. "Not a Californian species" according to Carpenter (1864:556), but tentatively placed in synonymy of *Siphonium lituella* Mörch, 1861, by Keen (1961:206). Tryon (1886:185, 440) referred to pl. 55, fig. 93 in his work; that figure does not exist. [Vermetidae]

melanostomus, Thylacodes, Mörch, 1865:99; "ad Zanzibar, in *Murice angulifero* Linn. affixum (Coll. O. Semper.) specimen unicum." Not located. [Vermetidae]

moebii [as *"möbii"*], *Tenagodus (Pyxipoma),* Mörch, 1865:98; "Hab. ----? ad Manillam? (Mus. Hamburg)" (p. 99). The dry collection of the Zoologisches Museum Hamburg was destroyed during World War II. The description alone is not sufficient for species identification. [Siliquariidae]

natalensis, Thylacodes, Mörch, 1862b:70; "Natal; specimen detritum communicavit T. Collins" (p. 71). Not located. [Vermetidae]

oryzata, Thylacodes?, Mörch, 1862b:78; "Litt. occid. Am. centralis verisimiliter; China (Mus. Cuming)." Mörch's doubts concerning Cuming's locality "China" were reiterated by Carpenter (1864:558) as "Probably W. Central America, from the adhesions," Tryon (1886:183) as "Panama," and Keen (1961:203) as "probably West Mexico." Holotype, BMNH 195912, *teste* Keen (1961:203, pl. 54, fig. 1). Keen (1961:203) placed this species in genus *Serpulorbis* and later (1983:10) gave the range as "La Paz, Baja California, to Acapulco." [Vermetidae]

pachylasma, Vermetus (Petaloconchus), Mörch, 1862a:354; "Guinea?"; "Royal Museum" (p. 355). Type specimen, ZMUC GAS-220, is the "dissected specimen" described by Mörch and could represent the holotype. "Probably a fossil" according to Tryon (1886:175). [Vermetidae]

pennatum, Stephopoma, Mörch, 1860a:43; Mörch distinguished between two varieties, a white-shelled Var. α from "Sur le *Crucibulum scutellatum* Gray. De Realejo," and a brown-shelled Var. β from "Sur le *Turbo (Uvanilla) saxosus*. Nord des îles Bocorones. OErsted." Mörch only described these "varieties," without addressing a nominate form. The two lots from the cited localities (ZMUC GAS-221 and GAS-222) are therefore considered syntypes. An additional lot from "Puntarenas & Realejo," ZMUC GAS-227, is considered possible type material. Subsequently Mörch (1861b:151, pl. 25, figs. 3-10) included "*Stephopoma bispinosum* Mörch" (see above) as a synonym. [Siliquariidae]

phillipsii, Burtinella, Mörch, 1861b:148; introduced for "*Vermicularia sowerbii* Mantell" *sensu* Phillips, 1829 (:124, pl. 2, figs. 29, 30; Mörch referred to the second edition of 1835 with slightly different pagination), non *Vermicularia sowerbii* Mantell, 1822; "Speeton Clay"; fossil. [Polychaeta?]

pictum, Siphonium Mörch, 1861b:161; "India Orientalis [East Indies]; in specimen pedale *Tridacnae gigantis* leviter corrodens ... (Mus. Regium)"; with one named variety. The *"S. pictum"* lot, ZMUC GAS-223, is on a *Tridacna* shell, but came from the

Nicobar Islands and can therefore probably not be considered as type material. Mörch (1862b:83) suggested that this (and two other) nominal species only represented "different ages of *S. subcrenatum*, Lam.," referring to the species described as *Vermilia subcrenata* Lamarck, 1818. [Vermetidae]

platypus, Siphonium (Stoa), Mörch, 1861b:157; "Sandwich [Hawaiian] Islands, on a *Chama.*" Holotype, BMNH 195927, *teste* Keen (1961:207). Species redescribed as *Dendropoma platypus* for the Hawaiian fauna by Hadfield *et al.* (1971:84). [Vermetidae]

ponderosus, Tenagodus (Siliquarius), Mörch, 1861a:409; "Port Essington [Northern Territory, Australia], 7 fathoms, sandy mud (Jukes); coll. Cuming. A single specimen." Holotype, BMNH 1995104. See Sowerby (1878:pl. 1, fig. 3 [copied in Tryon, 1886:57, fig. 11]; 1887, pl. 1 [480], fig. 5). [Siliquariidae]

rastrum, Vermiculus, Mörch 1861b:180; "(coll. Cuming.), sine loco" (p. 181). Lectotype, BMNH 195916, illustrated by Keen (1961:207, pl. 54, fig. 2; lectotype selection by inference of holotype). The two specimens from Puntarenas referred to by Mörch (1861b:181) are paralectotypes, ZMUC GAS-226. Keen (1961:207) noticed the operculum with the type as not congeneric and placed *V. rastrum*, based on the shell alone, in the vermetid genus *Dendropoma.* She regarded it as "probably Californian in origin." This is "*Vermiculum rostrum* Mörch" of Clessin (1903:84). [Vermetidae]

reentzii, Tenagodus, Mörch, 1865:98; "Australia (coll. Cuming); introduced for "*Tenagodus australis* Quoy & Gaimard var. δ ferruginea" *sensu* Mörch, 1861a:407. Holotype, BMNH 1995092. Sowerby (1878:pl. 4, fig. 12; 1887:pl. 2 [481], fig. 9) illustrated another specimen under this name, a high-spired morph described by Mörch (1861a:407) as "var. δ*" from South Australia that was not clearly included when the name was introduced in 1865. This is the species listed as *"Siliquaria Rensii"* and *"Rentsi"* by Clessin (1901:3; 1912:117). [Siliquariidae]

riisei, Thylacodes, Mörch, 1862b:69; "Ins. S. Thomae, Antillarum, Riise, Hornbeck (Mus. reg. et Univ.)"; including two named varieties (three specimens of "var. α limacella," BMNH 1986200). One syntype, ZMUC GAS-236, ex Hornbeck, plus four syntypes, ZMUC GAS-224, ex Riise; all from St. Thomas. This is "*Thylacodes Rüsei,* Mörch" of Carpenter (1864:557) and "*Serpulorbis (Thylacodes) Rüseii* Mörch" of Vaillant (1871). [Vermetidae]

scaphitella, Siphonium (Stoa), Mörch, 1861b:160; "Ins. Philippin.; a single detached specimen in Mr. Cuming's collection." Holotype, BMNH 1995105. Mörch (1862b:83) suggested that this (and two other) nominal species only represented "different ages of *S. subcrenatum,* Lam.," referring to the species described as *Vermilia subcrenata* Lamarck, 1818. [Vermetidae]

schroeteri, Spiroglyphus, Mörch, 1860a:45; "Val de Ronca"; based on Schröter's illustration (1780:pl. 1, fig. 2g). More detailed locality description was given subsequently by Mörch (1862a:330): "Fossil, Valle Canella in situ vulcanico di Ronca (Haquet). Burrowing on a *Strombus.*" Name here emended from "Schroteri," a spelling error in the French journal; it was clearly intended to be named for Schröter and was spelled "schröteri" by Mörch, 1862a:330. [Vermetidae]

senticosum, Stephopoma, Mörch, 1861b:150-151, pl. 25, figs. 2, 14; no locality given; "Mus. reg."; "in *Tridacna scapha* (Meusch.)." Possible syntypes, ZMUC GAS-225. Morton and Keen (1960:27) added "probably western Pacific." [Siliquariidae]

solarinus, Vermiculus Mörch, 1861b:171; "Ins. Philippin. (Cuming)." Holotype, BMNH 1995093, Philippine locality doubtful. [Turritellidae]

stramonitae, Spiroglyphus, Mörch, 1862a:330; "Guinea? on *Purpura (Stramonita) haemastoma,* L., var."; "Mus. Reg." Syntype lot, ZMUC GAS-228. [Vermetidae]

subgranosum, Siphonium, Mörch, 1861b:165; "India orientalis (Tranquebar ?)"; previously cited (Mörch, 1859:358) as "*Siphonium costale,* Lam."; including two named varieties. None of the ZMUC lots labelled with this name have locality information; they probably do not represent type material. [Vermetidae]

subtriquetra, Bivonia, Mörch, 1862b:58; including one named variety; locality information only given with that variety: "Fossilis in form. tertiaria ad Asti (coll. T.O. Semper)." Subsequently synonymized by Mörch (1865:99) under "*Vermetus articulatus,* Bonelli" as listed by Sismonda (1847:27). Not located. [Vermetidae]

sutilis, Bivonia, Mörch, 1862b:58; "In littore occidentali

Americae centralis, in valva solitaria *Veneris subimbricatae* Sow. affixa (Mus. Cuming)"; with two named varieties. Holotype, BMNH 197878. Carpenter (1864:557) paraphrased the locality information as [west coast of] "Central America, on *Anomalocardia subimbricata*." Keen (1961:209) placed the species in "?*Serpulorbis*," and later (1983:10) added "Gulf of California" locality. [Vermetidae]

tahitensis, Tenagodus (Pyxipoma), Mörch, 1861a:410; "Tahiti, coll. Cuming., specimen fractum, long. 6 cm." Holotype, BMNH 1995094. This is "*T. taheitensis,* Mörch" and "*Siliquaria taheitensis,* Mörch" of Tryon (1886:191 and 441, respectively), and "*Siliquaria tahietensis* ... Mörch" of Sowerby (1878:pl. 4, fig. 13; 1887:165). [Siliquariidae]

teredula, Siphonium (Dendropoma?), Mörch, 1861b:155; "On *Haliotis tuberculata*, probably from Morocco" (p. 156). Subsequently synonymized by Mörch (1871:129) as variety "? β" of "*Vermetus (Spiroglyphus) glomeratus* Biv. Phil., non L." Lot ZMUC GAS-229 is labelled *"Stoa teredula"* and came from Naples; it probably does not represent type material. [Vermetidae]

textum, Siphonium (Stoa?), Mörch, 1861b:159; "Ins. Philippin."; "H. Cuming. legit; extat in collectione Dr. Hornbeckii"; with two named varieties. Lot ZMUC GAS-230, from coll. Hornbeck ex Cuming, is labelled as from the Indian Ocean, not the "Philippines," and does probably not represent type material. Mörch (1862b:83) suggested that this (and two other) nominal species only represented "different ages of *S. subcrenatum*, Lam.," referring to the species described as *Vermilia subcrenata* Lamarck, 1818. [Vermetidae]

tostus, Tenagodus (Siliquarius), Mörch, 1861a:405; "Ins. Ceylon (E. L. Layard), specimen unicum." Holotype, BMNH 1995106. See Sowerby (1878:pl. 4, fig. 11 [copied in Tryon, 1886:pl. 57, fig. 18]; 1887:pl. 2 [481], fig. 10). [Siliquariidae]

tricuspe, Stephopoma, Mörch, 1861b:150, pl. 25, fig. 1; "Australia"; "coll. Cuming." Syntype cluster figured by Hedley (1913:294, pl. 19, figs. 72-74; shells and operculum from type material, BMNH 1995095 and 1995096). Hedley (1913:294) added: "it lives in Sydney Harbour." [Siliquariidae]

trochlearis, Tenagodus (Siliquarius), Mörch, 1861a:408; no locality indicated; "coll. Cumingii, specimen unicum." Four specimens including potential holotype, BMNH 1995107. See Sowerby (1878:pl. 2, fig. 4; 1887:pl. 2 [481], fig. 14) and Tryon (1886:pl. 57, fig. 14). Tryon (1886:189) gave "Philippines" as the locality. [Siliquariidae]

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LITERATURE CITED

Adams, A. 1860. On some new genera and species of Mollusca from Japan. *The Annals and Magazine of Natural History, including Zoology, Botany, and Geology* (3)5(18):299-303.

Adams, A. 1864. Notes on some molluscous animals from the Seas of China and Japan. *The Annals and Magazine of Natural History* (3)13:140-144.

Adanson, M. 1757. *Histoire naturelle du Sénégal. Coquillages. Avec la Relation abrégée d'un Voyage fait en ce Pays, pendant les Années 1749-53.* C.-J.-B. Bauche, Paris. viii + 190 + xcvi + 275 pp., 19 pls., 1 map.

Agassiz, L. 1848. *Nomenclatoris zoologici index universalis, continens nomina systematica classium, ordinum, familiarum et generum animalium omnium, tam viventium quam fossilium, secundum ordinem alphabeticum unicum disposita, adjectis homonymiis plantarum.* Jent and Gassmann, Soloduri. x + 1135 pp.

Bieler, R. 1992. *Tenagodus* or *Siliquaria*? Unraveling taxonomic confusion in marine "worm-snails" (Cerithioidea: Siliquariidae). *The Nautilus* 106(1):15-20.

Bivona-Bernardi, A. 1832. Continuazione dell' articolo sui vermeti, estartto dalle Collettanee di Storia naturale del barone Bivona. *Effemeridi scientifiche e letterarie per la Sicilia* 2(1):3-8.

Boettger, C. R. 1963. Die als Schalen juveniler Wurmschnecken (Fam. Vermetidae) angesprochenen Funde aus einer Höhle der Insel Kreta. *Archiv für Molluskenkunde* 92(1-2):77-78.

Boss, K. J. 1988. References to molluscan taxa introduced by Linnaeus in the *Systema Naturae* (1758, 1767). *The Nautilus* 102(3):115-122.

Browne, P. 1756. *The Civil and Natural History of Jamaica: in Three Parts.* Printed for the author and sold by T. Osborne and J. Shipton, London. viii + 503 pp., 49 pls. 1 map.

Browne, P. 1789. *The Civil and Natural History of Jamaica: in Three*

Parts, 2nd ed. [Cited by Keen, 1980; not seen].

Bucquoy, E., P. Dautzenberg, and G. Dollfus. 1884. *Les Mollusques marins du Roussillon* 1(6):223-257 (Famille IV ... Turritellidae Clark, 1851), pls. 28-30. [Also published in *Bulletin de la Société d'Études Scientifiques de Paris,* with different pagination.]

Carpenter, P. P. 1857a. First steps towards a monograph of the Recent species of *Petaloconchus*, a genus of Vermetidae. *Proceedings of the Zoological Society of London* 24(1856):313-317 [10 March 1857; *teste* Duncan, 1937].

Carpenter, P. P. 1857b. *Catalogue of the Collection of Mazatlan shells, in the British Museum: collected by Frederick Reigen.* Oberlin Press, Warrington. i-iv + ix-xvi + 552 pp. [Some copies have the pagination of introduction materials as "i-xii"; see Kabat, 1989.]

Carpenter, P. P. 1864. Supplementary report on the present state of our knowledge with regard to the Mollusca of the west coast of North America. *Report of the British Association for the Advancement of Science* for 1863:517-686. [Reprinted by Carpenter, 1872, as part of a larger compilation, *Smithsonian Miscellaneous Collections* 252:3-172.]

Clessin, S. 1901-1912. Die Familie Vermetidae. *Systematisches Conchylien-Cabinet von Martini und Chemnitz, 2nd ed.* (Küster and Kobelt) 6(6):1-24, pls. 1-12 (1901), 25-80, pl. 13 (1902), 81-104 (1903), pls. 14-15 (1904), 105-124 (1912) [including Caecidae, Siliquariidae, *Vermicularia*, and Vermetidae; dates *teste* Smith and England, 1937, and Johnson, 1968].

Collin, J. 1878. *Konchyliologen Otto Andreas Lowson Mørch. En biografisk Skizze.* Bianco Lunos Bogtrykkeri, Copenhagen. 38 pp. [portrait, biography, bibliography].

Cossmann, M. 1888. Catalogue illustré des coquilles fossiles de l'Eocene des environs de Paris. *Annales de la Société Royale Malacologique de Belgique* 23:1-324, pls. 1-12 [reprinted 1888, 328 pp., 12 pls., with slightly different pagination].

Cossmann, M. 1912. *Essais de Paléoconchologie comparée.* Privately published, Paris. Neuvième livraison:215 pp., 10 pls. [Scalidae by de Boury].

Costa, E. M. da. 1776. *Elements of Conchology: or, an Introduction to the Knowledge of Shells.* Printed for B. White, London. viii + 318 pp., 7 pls. [For a detailed account of this work and discussion of authorship, see Whitehead, 1977.]

Daudin, F. M. 1800. Mémoire sur le genre *Serpula* de Linné. *In: Recueil de Mémoires et de Notes sur des Espèces inédites ou peu connues de Mollusques, de Vers et de Zoophytes, orné de Gravures.* pp. 32-50, 2 pls. Fuchs, Treuttel and Wurtz, Paris. xviii + 50 pp., 4 pls.

Defrance, F. 1827. *Rotularia. In: Dictionnaire des Sciences Naturelles,* 46. Paris & Strasbourg. [not seen]

Della Campana, C. 1890. Cenni Paleontologici sul Pliocene antico di Borzoli. *Atti della Società Ligustica di Scienze Naturali e Geografiche* 1:128-165, pl. 4.

Deshayes, G. P. 1843. *Histoire naturelle des Animaux sans Vertèbres, presentant les Caractères généraux et particuliers de ces Animaux, leur Distribution, leurs Classes, leurs Familles, leurs Genres, et la Citation principales Espèces qui s'y rapportent ...,* 2nd ed. *9, Histoire des Mollusques.* J. B. Baillière, Paris. 728 pp.

Duncan, F. M. 1937. On the dates of publication of the Society's 'Proceedings,' 1859-1926. With an appendix containing the dates of publication of 'Proceedings,' 1830-1858, compiled by the late F. H. Waterhouse, and of the 'Transactions,' 1833-1869, by the late Henry Peavot, originally published in P. Z. S. 1893, 1913. *Proceedings of the Zoological Society of London* 107(A):71-84.

Dunker, W. 1860. Neue japanische Mollusken. *Malakozoologische* Blätter 6:221-240.

Dunker, G. [= W.]. 1861. *Mollusca japonica descripta et tabulis tribus iconum illustrata.* E. Schweizerbart, Stuttgart. iv + 36 pp., 3 pls.

Fischer, P. 1885. *Manuel de Conchyliologie et de Paléontologie conchyliologique ou Histoire naturelle des Mollusques vivants et fossiles (suivi d'un Appendice sur les Brachiopodes par D. Oehlert).* F. Savy, Paris. 8:689-784 (1885); atlas of 23 pls. (undated).

Fischer-Piette, E. ("avec collaboration de P.-H. Fischer, L. Germain et P. Pallary"). 1942. Les mollusques d'Adanson. *Journal de Conchyliologie* 85:101-366, pls. 1-16.

Gardner, J. 1939. Notes on fossils from the Eocene of the Gulf Province. I. The annelid genus *Tubulostium.* II. The gastropod families Cassididae, Ficidae, and Buccinidae. *United States Geological Survey Professional Paper* 139-B:17-44, pls. 6-8.

Gmelin, J. F. 1791. *Caroli a Linné ... Systema naturae per regna tria naturae,* 13th ed. Leipzig. 1(6), Vermes: 3021-3910.

Gray, J. E. 1842. [Mollusca] *In: Synopsis of the Contents of the British Museum, 44th ed.* pp. 48-92. G. Woodfall and Son, London. [iv] + 308 pp.

Gray, J. E. 1847. A list of the genera of Recent Mollusca, their synonyma and types. *Proceedings of the Zoological Society of London* for 1847:129-219.

Gray, J. E. 1850. Explanations of plates and list of genera. *In: Figures of Molluscous Animals, Selected from Various Authors. Etched for the Use of Students, Vol. 4,* by M. E. Gray. 124 pp. Longman, Brown, Green and Longman, London.

Gray, M. E. 1850. *Figures of Molluscous Animals, Selected from Various Authors. Etched for the Use of Students, Vol. 4.* Longman, Brown, Green and Longman, London. 312 pls.

Gronovius, L. T. 1781. *Zoophylacii Gronoviani fasciculus tertius, exhibens vermes, mollusca, testacea, et zoophyta, quae in Museo suo adservavit, examini subjecit, systematice disposuit atque descripsit.* T. Haak and Soc., S. and J. Luchtmans, Lugduni Batavorum [Leiden]. [8] + 241-380 pp., pls. 18-20 [= part 3, pls. 1-3]. [Rejected for nomenclatural purposes, ICZN, 1954.]

Guettard, J. É. 1770. *Mémoires sur différentes Parties des Sciences et Arts, 3.* L. Prault, Paris. [seen partial copy only]

Hadfield, M. G., E. A. Kay, M. U. Gillette, and M. C. Lloyd. 1971. The Vermetidae (Mollusca: Gastropoda) of the Hawaiian Islands. *Marine Biology* 12(1):81-98.

Hedley, C. 1913. Studies on Australian Mollusca. Part XI. *Proceedings of the Linnean Society of New South Wales* 38(2):258-339, pls. 16-19.

ICZN [International Commission on Zoological Nomenclature]. 1954. Opinion 261. Rejection for nomenclatural purposes of the Index to the *Zoophylacium Gronovianum* of Gronovius prepared by Meuschen (F. C.) and published in 1781. *Opinions and Declarations Rendered by the International Commission on Zoological Nomenclature* 5(22):281-296, 1 facsimile.

ICZN. 1958. Addition to the "Official Index of Rejected and Invalid Generic Names in Zoology" of two further junior homonyms of *"Vermiculus"* Dalyell, 1853. *Opinions and Declarations Rendered by the International Commission on Zoological Nomenclature,* Direction 102:214-215.

ICZN. 1985. *International Code of Zoological Nomenclature, 3rd ed.* International Trust for Zoological Nomenclature and British Museum [Natural History], London, and University of California Press, Berkeley and Los Angeles. xx + 338 pp.

ICZN. 1987. Opinion 1425. Suppressed: *Spiroglyphus* Daudin, 1800 and *Stoa* De Serres, 1855 (Mollusca, Gastropoda) and specific names published in combination with them. *Bulletin of Zoological Nomenclature* 44(1):57-58.

Iredale, T. 1937. Middleton and Elizabeth Reefs, South Pacific Ocean.

Australian Zoologist 8(4):232-261, pls. 15-17.

Iwakawa, T. 1909. *Catalogue of Japanese Mollusca in the Natural History Department, Tokyo Imperial Museum. Part I. (Marine Gasteropoda including Scaphopoda).* Tokyo Imperial Museum, Tokyo. 135 + 16 + 13 pp.

Janssen, R. 1993. Die Typen und Typoide des Natur-Museums Senckenberg, 81. Die Typen der von Dunker 1860/1861 beschriebenen japanischen Meeresmollusken. *Archiv für Molluskenkunde* 122:403-435.

Johnson, R. I. 1968. Martini and Chemnitz (Kuester's edition) *Systematisches Conchylien-Cabinet,* 1837-1920, a complete collation. *Journal of the Society for the Bibliography of Natural History* 4(7):363-367.

Kabat, A. R. 1989. The "Gray Catalogues" [Mollusca] of the British Museum. *The Nautilus* 103(3):113-115.

Keen, A. M. 1961. A proposed reclassification of the gastropod family Vermetidae. *Bulletin of the British Museum (Natural History), Zoology* 7(3):183-213, pls. 54-55.

Keen, A. M. 1966. Moerch's west Central American molluscan types with proposal of a new name for a species of *Semele. Occasional Papers of the California Academy of Sciences* 59:1-33.

Keen, A. M. 1971. *Sea Shells of Tropical West America; Marine Mollusks from Baja California to Peru, 2nd ed.* Stanford University Press, Stanford, California. xiv + 1064 pp., 22 pls.

Keen, A. M. 1973. Suggested generic allocations for some Japanese molluscan species. *Tohoku University, Science Reports, 2nd series (Geology), Special Volume 6* (Hatai Memorial Volume):1-6.

Keen, A. M. 1980. *Siphonium*, an over-used name in Mollusca. *The Festivus* 12(1):125-126.

Keen, A. M. 1983. Vermetidae of the tropical eastern Pacific. *The Western Society of Malacologists Annual Report* 15:10.

Kuroda, T. and T. Kinoshita. 1951. Icones of marine animals and plants of Hokkaidô. Mollusca No. 1. A catalogue of marine molluscan shells of Hokkaidô. *Bulletin of Hokkaido Regional Fisheries Research Laboratory Fisheries Agency* 2:1-40 (in Japanese).

Lamarck, [J. B. P. A. de Monet de]. 1799. Prodrome d'une nouvelle classification des coquilles, comprenant une rédaction appropriée des caractères génériques, et l'établissement d'un grand nombre de genres nouveaux. *Mémoires de la Société d'Histoire Naturelle de Paris* 1:63-91.

Lamarck, J. B. [P. A. de Monet de]. 1801. *Systême des Animaux sans Vertèbres, ou Tableau général des Classes, des Ordres et des Genres de ces Animaux...* Lamarck and Deterville, Paris. viii + 432 pp.

Lamarck, [J. B. P. A. de Monet] de. 1818. *Histoire naturelle des Animaux sans Vertèbres..., 5.* Deterville and Verdiere, Paris. 612 pp.

Lamarck, [J. B. P. A. de Monet] de. 1822. *Histoire naturelle des Animaux sans Vertèbres..., 6(2).* Lamarck, Paris. 232 pp.

Linné [Linnaeus], C. 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis ... Editio decima reformata.* Stockholm. 1:824 pp. [Vermes Testacea:667-788].

Link, H. F. 1807. *Beschreibung der Naturalien-Sammlung der Universität zu Rostock. Vierte Abtheilung.* Adlers Erben, Rostock. Mollusken: pp. 6-23.

Lister, M. 1688. *Historiae Conchyliorum, Liber IV, qui est de Buccinis Marinis, etiam Vermiculj, Dentalia, et Patellae numerantur, Sectio III, de Vermiculis.* London. [For collations, see Wilkins, 1957, and Boss, 1988]

Mantell, G. 1822. *The Fossils of the South Downs; or Illustrations of the Geology of Sussex.* London. xvi + 327 pp., 42 pls.

Marschall, A. de. 1873. *Nomenclator zoologicus continens nomina systematica generum animalium tam viventium quam fossilium, secundum ordinem alphabeticum disposita.* C. Ueberreuter, Vienna. vi + 482 pp.

Martens, E. von. 1860. *Die Heliceen, nach natürlicher Verwandtschaft systematisch geordnet von J. Chr. Albers. Zweite Ausgabe nach dem hinterlassenen Manuscript besorgt von Eduard von Martens.* Wilhelm Engelmann, Leipzig. xviii + 359 pp.

Mayer[-Eymar], C. [= K.]. 1860. Description d'un genre nouveau de Protopodes. *Journal de Conchyliologie* 8(3):308-310 [July according to volume and *teste* Winckworth, 1936; cited as "August" by Mörch, 1861b:147].

Mermod, G. and E. Binder. 1963. Les types de la Collection Lamarck au Muséum de Genève. Mollusques vivants. V. *Revue Suisse de Zoologie* 70(7):127-172.

Mörch, O. A. L. 1859. Études sur la famille des Vermets. *Journal de Conchyliologie* 7(4):342-360 [June] [separately paged 1-40].

Mörch, O. A. L. 1860a. Études sur la famille des Vermets (suite). *Journal de Conchyliologie* 8(1):27-48 [January].

Mörch, O. A. L. 1860b. Beiträge zur Molluskenfauna Central-Amerika's. *Malakozoologische Blätter* 7(für 1860):66-96 [July], 97-106 [August].

Mörch, O. A. L. 1860c. Description d'une espèce nouvelle *[Tenagodus (Siliquarius) bernardii]. Journal de Conchyliologie* 8(4):368-369 [October].

Mörch, O. A. L. 1861a. Review of the genus *Tenagodus*, Guettard. *Proceedings of the Zoological Society of London* 1860, part 3:400-415. (Duncan [1937:72] dated this part as "issued between August 1860 and March 1861"; because this particular paper was presented at the session of 13 November 1860, a publication date in early 1861 is here assumed).

Mörch, O. A. L. 1861b. Review of the Vermetidae. Part I. *Proceedings of the Scientific Meetings of the Zoological Society of London* for the year 1861, part 2:145-181, pl. 25 [September] [separately paged 1-37, pl. 25].

Mörch, O. A. L. 1862a. Review of the Vermetidae. Part II. *Proceedings of the Scientific Meetings of the Zoological Society of London* for the year 1861, part 3:326-365 [April] [separately paged 1-41].

Mörch, O. A. L. 1862b. Review of the Vermetidae. Part III. *Proceedings of the Scientific Meetings of the Zoological Society of London* for the year 1862, part 1:54-83 [June] [separately paged 1-31].

Mörch, O. A. L. 1863. Revisio critica Serpulidarum, et Bidrag til Rørormenes Naturhistorie. *Naturhistorisk Tidsskrift* (3)1(3):347-470, pl. 11.

Mörch, O. A. L. 1865. Supplementary notes to the review of Vermetidae. *Proceedings of the Scientific Meetings of the Zoological Society of London* for the year 1865, part 1:96-99 [June].

Mörch, O. A. L. 1871. Uebersicht der europäischen Vermetiden. *Malakozoologische Blätter* 18(für 1871):128-131.

Mörch, O. A. L. 1877. Synopsis molluscorum marinorum Indiarum occidentalium imprimis Insularum danicarum [continued]. *Malakozoologische Blätter* 24 (für 1877):93-123.

Monterosato, T. [di M.] Marchese di. 1892. Monografia dei Vermeti del Mediterraneo. *Bulletino della Società Malacologica Italiana* 17:7-48, pls. 1-7.

Morton, J. E. and A. M. Keen. 1960. A new species of *Stephopoma* (Siliquariidae: Mesogastropoda) from the eastern Atlantic Ocean. *Proceedings of the Malacological Society of London* 34(1):27-35, pl. 1.

Oldroyd, I. S. 1927. The marine shells of the west coast of North America. *Stanford University Publications, University Series, Geological Sciences* 2(3):1-339 [603-941], pls. 73-108.

Philippi, R. A. 1836. *Enumeratio molluscorum Siciliae cum viventium tum in tellure tertiaria fossilium, quae in itinere suo observavit.*

S. Schroppii and Soc., Berlin. 267 pp., 12 pls.

Philippi, R. A. 1846-1847. Verzeichniss der in der Gegend von Magdeburg aufgefundenen Tertiärversteinerungen. *Palaeontographica* 1(1):42-44 (1846), 1(2):45-90, pls. 7-10.

Phillips, J. 1829. *Illustrations of the Geology of Yorkshire; or, a Description of the Strata and Organic Remains of the Yorkshire Coast: Accompanied by a Geological Map, Sections, and Plates of the Fossil Plants and Animals.* J. Phillips, York. [seen partial copy only]

Pilsbry, H. A. 1892. New and hitherto unfigured Japanese mollusks. *Proceedings of the Academy of Natural Sciences of Philadelphia* 43(1891):471-473, pls. 17-19.

Quoy, J. R. C. and J. P. Gaimard. 1834. *Voyage de Découvertes de l'Astrolabe exécuté par Ordre du Roi, pendant les Années 1826-1827-1828-1829, sous le Commandement de M. J. Dumont d'Urville. Zoologie, Mollusques.* 3(1):1-366 (1834); atlas, pls. 1-107 [dates of plates not verified]. J. Tastu, Paris.

Rovereto, G. 1899. Prime recerche sinonimiche sui generi dei gasteropodi. *Atti della Società Ligustica di Scienze Naturali e Geografiche* 10:101-110.

Ruhoff, F. A. 1980. Index to the species of Mollusca introduced from 1850 to 1870. *Smithsonian Contributions to Zoology* 294:1-640.

Sacco, F. 1896. *I molluschi dei terreni terziarii del Piemonte e della Liguria. Parte XX (Caecidae, Vermetidae, Siliquariidae, Phoridae, Calyptraeidae, Capulidae, Hipponycidae, Neritidae e Neritopsidae).* C. Claussen, Turin. 65 pp., 5 pls.

Sacco, F. 1904. *I molluschi dei terreni terziarii del Piemonte e della Liguria. Parte XXX. Aggiunte e correzioni, considerazioni generali, indice generale dell' opera.* C. Clausen, Turin. 203 + xxxvi pp., 31 pls.

Sandberger, C. L. F. 1859. *Die Conchylien des Mainzer Tertiärbeckens.* C. W. Kreidel, Wiesbaden. Issue 3 (sheets 10-14):73-112, pls. 11-15. [Date after Schönberg, 1907.]

Sasso, A. 1827. Saggio geologico sopra il Bacino terziario di Albenga. *Giornale Ligustico di Scienze, Lettere, ed Arti* 1(5):467-484. [The author's name is Sasso, not "Sassi" as often cited; see Monterosato, 1892:8.]

Schlesch, H. 1943. Otto Andreas Lowson Mörch (1828-1878). *Archiv für Molluskenkunde* 75(4):214-225.

Schönberg, F. 1907. Verzeichnis der im Naturhistorischen Museum zu Wiesbaden aufbewahrten Originale. Abteilung für Geologie und Paläontologie. 1. Originale zu Frid. Sandberger, Die Konchylien des Mainzer Tertiärbeckens. *Jahrbücher des Nassauischen Vereins für Naturkunde in Wiesbaden* 60:148-169.

Schmidt, W. J. 1955. Nomenklatur und Systematik der Serpuliden-Gattung *Rotularia* Defrance (=*Tubulostium* Stoliczka). *Mitteilungen der Geologischen Gesellschaft in Wien* 47(1954):159-182, 1 pl.

Schröter, J. S. 1780. *Journal für die Liebhaber des Steinreichs und Konchyliologie* 6.

Sismonda, E. 1847. *Synopsis methodica animalium invertebratorum pedemontii fossilium (exceptis speciebus ineditis), ed. 2.* Typis Regiis, Augustae taurinorum [Turin]. viii + 62 pp.

Smith, E. A. and H. W. England. 1937. Martini and Chemnitz (Kuester's edition) Systematisches Conchylien-Cabinet, 1837-1918. *Journal of the Society for the Bibliography of Natural History* 1(4):89-99.

Sowerby, G. B. 1878. Monograph of the genus *Siliquaria. Conchologia Iconica: or, Illustrations of the Shells of Molluscous Animals* 20:5 unnumbered pp., 4 pls.

Sowerby, G. B. 1887. Principal species of the genus *Siliquaria*, Brug. *Thesaurus Conchyliorum, or Monographs of Genera of Shells* 5:163-165, pls. 1-2 (= Thesaurus pls. 480-481).

Stimpson, W. 1851. *Shells of New England. A Revision of the Synonymy of the Testaceous Mollusks of New England, with Notes on their Structure, and their Geographical and Bathymetrical Distribution.* Phillips, Sampson and Company, Boston. 57 pp., 2 pls.

ten Hove, H. A. 1994. The dualistic relation between molluscs and serpulid tube-worms. *In: De horen en zijn echo*, M. Coomans-Eustatia, R. Moolenbeek, W. Los, and P. Prins, eds. pp. 65-70. Stichting Libri Antilliani, Zoölogisch Museum Amsterdam. 279 pp.

ten Hove, H. A. and P. van den Hurk. 1993. A review of Recent and fossil serpulid 'reefs'; actuopalaeontology and the 'Upper Malm' serpulid limestones in NW Germany. *Geologie en Mijnbouw* 72:23-67.

Turton W. H. 1932. *The Marine Shells of Port Alfred S. Africa.* Oxford University Press, London. xvi + 331 pp., 70 pls.

Tryon, G. W. 1886. *Manual of Conchology; Structural and Systematic. With Illustrations of the Species. Vol. VIII. Naticidae, Calyptraeidae, Turritellidae, Vermetidae, Caecidae, Eulimidae, Turrbonillidae, Pyramidellidae.* G. W. Tryon, Philadelphia. 461 pp., 79 pls.

Vaillant, L. 1871. Recherches sur la synonymie des espèces placées par de Lamarck dans les genres Vermet, Serpule, Vermilie, et appartenant a la famille des Tubispirata. *Nouvelles Archives du Muséum d'Histoire Naturelle de Paris* 1871:181-201.

Wenz, W. 1938-1944. Gastropoda, Teil I: Allgemeiner Teil und Prosobranchia. *In: Handbuch der Paläozoologie,* 6, O. H. Schindewolf, ed. xii + 1639 pp. [Teil 3, pp. 481-720, published July 1939]. Bornträger, Berlin.

Whitehead, P. J. P. 1977. Emanuel Mendes da Costa (1717-91) and the *Conchology, or natural history of shells. Bulletin of the British Museum (Natural History), Historical Series* 9(1):1-24.

Wilkins, G. L. 1957. Notes on the "Historia Conchyliorum" of Martin Lister (1683-1712). *Journal of the Society for the Bibliography of Natural History* 3(4):196-205, portrait.

Winckworth, R. 1936. Journal de Conchyliologie: dates of publication. *Proceedings of the Malacological Society of London* 22(3):153-156.

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Nurse-egg feeding prosobranchs: a comparative biochemical and electrophoretic analysis of eggs and hatchlings

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Abstract: A biochemical study of the eggs and hatchlings of cold-temperate and arctic-boreal (*Buccinum undatum* Linné, 1758, and *B. cyaneum* Bruguière, 1792, respectively) and tropical (*Fasciolaria tulipa hollisteri* Weisbord, 1962, *Fusinus closter* Philippi, 1850, and *Eualetes tulipa* Chenu, 1843) nurse-egg feeding gastropods was carried out to determine if the total protein, glycogen, and lipid contained in eggs and nurse eggs were enough to account for the same totals in the hatchlings. An electrophoretic study was also conducted on the eggs and hatchlings of the five species in order to compare qualitatively the patterns of egg proteins and to determine how much of these were converted to other molecular weight proteins in the hatchling. Biochemical analysis indicated that the buccinids and the vermetid had more-or-less enough material in eggs and nurse eggs to account for the total in the hatchling. However, fasciolariids did not have sufficient material in eggs and nurse eggs to account for the hatchlings, so some of the material must come from another source within the egg capsule. Different families exhibited different patterns of electrophoretic egg proteins and all species converted most of the high molecular weight (HMW) egg proteins to different low molecular weight (LMW) proteins with the exception of *F. t. hollisteri*, which retained most of the HMW proteins. This study indicates that there are taxonomic differences at the family level in the biochemical construction of the hatchlings and in the electrophoretic pattern of the eggs.

Key words: biochemistry, electrophoresis, development, prosobranchs, nurse eggs

Prosobranch gastropods present different strategies during early development. Shuto (1974) pointed out that the adaptation of prosobranchs in the production of eggs can be divided into two strategies. The first maximizes the number of survivors during this period by spawning a large number of eggs, while the other strategy minimizes the wastage or early mortality by providing the embryos special protection from the environment. One such way of protection is by enclosing the eggs within structurally and chemically complex and energetically costly encapsulating structures or egg capsules (Pechenik, 1986).

The benefits associated with encapsulating structures are suggested to be protective (against physical stress, predators, and bacterial attack) and nutritional, given that the egg capsule can contain nurse eggs, "nurse yolk," or the fluid itself could be nutritive (Pechenik, 1986). Encapsulated embryos can meet their nutritional needs in four ways: (1) by feeding on the yolk of the egg from which they develop; (2) on proteins, sugars and other substances contained in the capsular fluid (Fioroni, 1966; Fioroni and Portmannn, 1968; De Mahieu *et al.*, 1974; Harasewych, 1978; Stockmann-Bosbach and Althoff, 1989; Penchaszadeh and Rincón, 1996); (3) by cannibalism or ingestion of sibling embryos; and (4) by adelphophagy or ingestion of nurse eggs. The hatching mode, planktotrophic or lecithotrophic larvae or crawling juveniles, will vary depending on the amount of food available during early development.

These extraembryonic food sources and particularly nurse eggs provide the embryo with the neccesary nutrients and energy to complete development to the hatching stage and in some cases, provide the hatchling with sufficient reserves to grow and survive during a period where food availability can be limited. The hatchlings of the nurse-egg feeding prosobranch *Nucella lapillus* Linné 1758, can survive up to 120 days without food indicating that the storage of nurse eggs can be crucial for survival (Gosselin and Chia, 1994). The importance of nurse eggs in the development of prosobranchs has been reviewed in 113 nurse-egg feeding species by Fioroni (1988). In this work, Fioroni described the importance of different modes of ingestion of the nurse eggs as well as the adaptations of the larvae for the management of these eggs. A recent review pertaining to why these eggs do not develop was given by Miloslavich and Dufresne (1994).

Within the Buccinidae, in the genera *Buccinum* and *Buccinanops* (restricted to temperate northern and temperate southern localities, respectively), nurse eggs or undeveloped eggs have been reported in several species, hatching taking place as crawling juveniles (Portmann, 1925; Thorson, 1935; Fretter and Graham, 1962; Penchaszadeh, 1971a, b, 1973; Martel *et al.*, 1986). Tropical buccinids, generally, do not have nurse eggs and hatch as veliger larvae or as crawling juveniles (Bandel, 1975, 1976; Miloslavich and Penchaszadeh, 1994).

Among fasciolariids, all studied *Fasciolaria* species ingest nurse eggs and hatch as crawling juveniles with the exception of *F. trapezium* Linné, 1758, which hatches as a veliger larva (Glaser, 1905; Hyman, 1923, 1935; Gohar and Eisawy, 1967; D'Asaro, 1970, 1986; Penchaszadeh and Paredes, in press). In *Fusus* and *Fusinus* species, nurse eggs have been reported by Amio (1963) in *Fusinus perplexus* A. Adams, 1864; in *Fusinus closter* Philippi, 1850, hatching takes place as crawling juveniles (Miloslavich and Penchaszadeh, in press).

Within the vermetid family, nurse eggs have been reported in several species (Hadfield, 1970, 1989; Hadfield *et al.*, 1972, Hughes, 1978; Hadfield and Iaea, 1989; Miloslavich and Penchaszadeh, 1992). Most of the species hatch as crawling juveniles with few exceptions, which hatch as veliger larvae.

The biochemical changes from egg to hatching in nurse-egg feeding species have been studied by Miloslavich and Dufresne (1994) in the buccinids *Buccinum cyaneum* Bruguière, 1792, and *B. undatum* Linné, 1758. These authors reported an increase of 100-fold in the protein and lipid amounts and of 250-fold in the glycogen amount per embryo during development. The question is whether all of this increase in the biochemical content of the hatchling takes place only by ingesting the nurse eggs.

We conducted a biochemical study of the eggs and hatchlings of cold-temperate and arctic-boreal buccinids and tropical fasciolarids and vermetid nurse-egg feeding species. The purpose was to determine if the total protein, glycogen, and lipid contained in eggs and nurse eggs are enough to account for the same totals in the hatchlings. An electrophoretic study was also conducted on the eggs and hatchlings of the same species in order to qualitatively compare the patterns of egg proteins and to determine how much of these were converted to other molecular weight proteins in the hatchling. The hypothesis is that if the densities of the egg proteins of the hatchling are similar to those of the egg, it would indicate that the egg material was not completely metabolized by the embryos, confirming the need of other food sources besides nurse eggs during intracapsular development.

MATERIALS AND METHODS

Specimens

The temperate species used in this study were the buccinids *Buccinum undatum* and *B. cyaneum*. *B. cyaneum* specimens were collected subtidally (20-40 m depth) in July 1991 with the help of baited cages, at the southwestern side of the Anse St. Jean, Saguenay Fjord, Quebec, Canada. *B. undatum* specimens were collected in the same way in July 1991 at Rivière de La Madeleine, St. Lawrence Estuary, Canada. Groups of snails of each species were kept in different troughs at the Pointe-au-Pere Aquaculture Station and supplied with sea water pumped from the St. Lawrence Estuary. The temperature varied from 5 to 10°C and salinities from 28 to 32 ppt. They were fed twice a week with frozen fish. A total of 26 spawn masses (from 26 different females) of *B. cyaneum* were obtained between August and November 1991 and only one spawn (one female) of *B. undatum* was obtained in July 1991 in the laboratory.

The tropical species were *Fasciolaria tulipa hollisteri* Weisbord, 1962, *Fusinus closter*, and *Eualetes tulipa* Chenu, 1843. The fasciolariids *F. t. hollisteri* and *F. closter* were collected subtidally (0.6-4.0 m depth) in February 1992, at Isla Caribe, Chacopata, northern Araya Peninsula, Estado Sucre, Venezuela. A total of three spawn masses of *F. t. hollisteri* were collected in the field found attached to hard substrata, such as empty *Pinna* or other bivalve shells. The spawn masses of *F. closter* were obtained both in the field (two spawns) and in the laboratory (three spawns) within the same week that the adults were collected from the field. They were kept with the *F. t. hollisteri* spawns in aquaria at the Marine Biology Laboratory, Universidad Simón Bolívar, at a temperature of 25-27°C.

The vermetid *Eualetes tulipa* was collected intertidally and subtidally in February 1992, in Morón, Estado Carabobo, Venezuela, where it was found attached to the walls of the Planta Centro power plant cooling-water intake channel. This species corresponds to the same species identified as *Vermetus* sp. in Miloslavich and Penchaszadeh (1992). A total of ten adults were kept in aquaria at a temperature of 25-27°C. The egg capsules were taken directly from the mantle cavity of the female. Each female brooded up to 25 egg capsules at different stages of development.

Biochemical Procedures

The eggs and hatchlings were obtained by carefully opening the egg capsules. Samples of eggs and hatchlings were placed in 1.5 ml Eppendorf tubes. All eggs or hatchlings contained within one Eppendorf tube came from the same capsule. The Eppendorf tubes with the samples were centrifuged at 30 rpm for ten seconds and excess water was aspirated. To determine the protein content of eggs and juveniles of the five species, a Bio-Rad protein micro-assay procedure based on the Bradford method was carried out (Bradford, 1976). Bovine serum albumin (BSA) was used as a standard. The samples were left overnight in 0.5 N NaOH at 4°C and thoroughly homogenized. The glycogen and lipid determinations were done following the methodology described in Miloslavich and Dufresne (1994).

Table 1. Number of egg and hatchling samples used in the electrophoretic procedure and quantity of protein introduced in each electrophoretic lane.

Species	# Egg samples	Eggs/ sample	Egg protein/ lane (μg)	# Hatchling samples	Hatchlings/ sample	Hatchling protein/ lane (μg)
Buccinum undatum	4	100-300	15	2	1	15
B. cyaneum	17	100-300	15	11	2-4	15
Fasciolaria t. hollisteri	2	600	10	2	2-3	20
Fusinus closter	5	20-100	20	3	2-3	20
Eualetes tulipa	17	100	20	3	60-70	20

Electrophoretic Procedure

To determine the number and molecular weight of the proteins composing the eggs and hatchlings, a qualitative analysis was done by electrophoresis on sodium dodecyl sulphate - polyacrylamide gels (SDS-PAGE), according to the procedure of Laemmli (1970). Samples were digested in Sample Buffer 1X (SB = upper gel buffer 4/10, SDS 8%, mercapto-ethanol 20%, glycerol 40% and brom phenol blue 0.008%); the volume of SB added to the sample was 1 μl per μg of protein in the sample. Samples were run on gels containing 10% acrylamide. The gels were stained with Coomasie blue and scanned with the Ultroscan XL Enhanced Laser Densitometer. Density readings were carried out with the GelScan XL Pharmacia LKB Biotechnology (ver. 2.1) software. For *Fasciolaria tulipa hollisteri*, the densities obtained at the hatching stage were reduced by half, given that the amount of protein contained in lane c' was twice the amount contained in lane c. Proteins representing more than 10% each of the total electrophoretic bands were considered to be major, while those consisting between 3-10% each of the total were considered to be minor. The number of egg and hatchling samples, the number of eggs and hatchlings per sample, as well as the quantity of protein introduced in each electrophoretic lane are specified for each species in Table 1.

RESULTS

The reproductive aspects of the five species studied are summarized in Table 2.

Biochemical Content

The protein content of the eggs of the five species was very similar, varying between 1.9 and 3.0 μg per egg. The glycogen and lipid content of the eggs was also similar

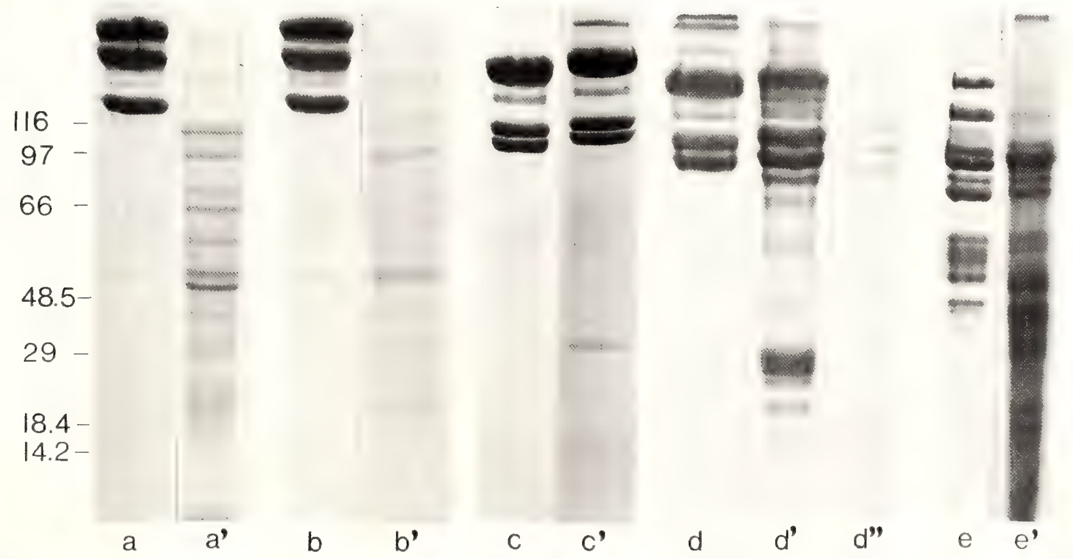

Fig. 1. SDS-PAGE (10%) electrophoresis of eggs and hatchlings. 1a. *Buccinum undatum*, egg stage. 1a'. *B. undatum*, hatching stage. 1b. *B. cyaneum*, egg stage. 1b'. *B. cyaneum*, hatching stage. 1c. *Fasciolaria tulipa hollisteri*, egg stage. 1c'. *F. t. hollisteri*, hatching stage. 1d. *Fusinus closter*, egg stage. 1d'. *F. closter*, disintegrated nurse eggs. 1d''. *F. closter*, hatching stage. 1e. *Eualetes tulipa*, egg stage. 1e'. *E. tulipa*, hatching stage.

Table 2. Information on some reproductive aspects of the five prosobranch species studied. Values represent mean ± standard deviation. (N = sample size).

Species	Eggs/ capsule	Egg diameter (μm)	Hatchling/ capsule	Hatchling shell length (mm)	Egg : Hatchling ratio	Period of development Hatching mode Temperature
Buccinum undatum	918 ± 256 N = 4	260 ± 10 N = 69	10 ± 5 N = 10	2.6	92 : 1	6 months Crawling 5-10°C
B. cyaneum	567 ± 227 N = 76	240 ± 20 N = 265	3 ± 2 N = 83	2.46 ± 0.33 N = 182	189 : 1	9 months Crawling 5-10°C
Fasciolaria t. hollisteri	3119 ± 908 N = 50	251 ± 17 N = 100	7 ± 2 N = 9	4.4 ± 0.6 N = 13	446 : 1	± 3 months Crawling 25-27°C
Fusinus closter	291 ± 73 N = 9	260 ± 19 N = 29	17 ± 8 N = 39	1.61 ± 0.13 N = 24	17 : 1	6-7 weeks Crawling (velum remains) 25-27°C
Eualetes tulipa	289 ± 114 N = 33	240 ± 14 N = 401	188 ± 87 N = 4	0.45 ± 0.02 N = 55	1.5 : 1	20 days Veliger 25-27°C

between species with the exception of *Eualetes tulipa* which had a much higher content of these substances (Table 3). All species increased dramatically the protein, glycogen, and lipid contents from the egg to the hatching stage; the exception was *E. tulipa* which decreased the protein content from the egg to the hatching stage and increased only around 1.5 fold the glycogen and lipid content.

Buccinid species had enough protein in eggs and nurse eggs to account for all protein in the hatching juvenile. However, they did not have sufficient glycogen in eggs and nurse eggs to account for the hatchlings. In the case of lipids, the eggs and nurse eggs of *Buccinum cyaneum* had enough to account for the hatchling but those of *B. undatum* did not. The final balance was positive for *B. cyaneum*, that is, there was sufficient protein, glycogen, and lipid in the eggs and nurse eggs to account for the hatching embryo. This balance was slightly negative for *B. undatum* (Table 4).

Fasciolariids did not have sufficient protein, glycogen, and lipid in eggs and nurse eggs to account for all of these materials in the hatchlings, the final balance for both species was negative being the total increase of three fold.

The vermetid had more than sufficient protein in eggs and nurse eggs to account for the hatchlings in the case of glycogen and lipid, the quantities contained at the egg and hatching stage were well balanced. The final balance was more-or-less equal.

Electrophoretic Patterns

For all species, eggs were mostly constituted by high molecular weight (HMW) proteins (more than 100 Kd) while hatchlings were constituted by several low molecular weight (LMW) and some HMW proteins. The hatchling of *Fasciolaria tulipa hollisteri* seemed to be mostly constituted by HMW proteins (Fig. 1, Table 5). No intraspecific variation in the protein pattern was found between samples of eggs and hatchlings (not proven in *Buccinum undatum* in which both samples belonged to the same female).

The egg patterns of both buccinids were almost identical (Figs. 1a, b). Fasciolariid eggs showed similar proteins but there were also clear differences between the two species (Figs. 1c, d). The vermetid egg proteins were different from those of the other two families (Fig. 1e).

Buccinids converted most HMW proteins to LMW proteins (Figs. 2a, a', b, b'). The fasciolariid *Fasciolaria tulipa hollisteri* retained most of the egg proteins (Figs. 2c, c') and *Fusinus closter* converted most of the HMW egg proteins into LMW proteins (Figs. 2d, d', d''). The vermetid also converted almost all HMW egg proteins to LMW proteins. This indicates that all species metabolized almost all of the HMW proteins during development to the hatching stage with the exception of *F. t. hollisteri* which used very few of the egg proteins.

DISCUSSION

Biochemical Content

Fioroni (1982) stated that molluscan development is influenced by phylogenetic recapitulations and by cenogenetic factors such as the diameter of the egg and the amount of extraembryonic food reserves. Hadfield (1989)

Table 3. Protein, glycogen, and lipid content per egg and per hatchling of the five prosobranch species studied (µg/individual). Values represent mean ± standard deviation. (N = sample size).

Species	Protein		Glycogen		Lipid	
	Egg	Hatchling	Egg	Hatchling	Egg	Hatchling
Buccinum undatum	3.0 ± 1.1 N = 4	106.8 ± 42.4 N = 2	5.4 ± 3.8 N = 4	688.1 ± 81.6 N = 5	2.1 ± 0.6 N = 3	625.2 ± 263.2 N = 6
B. cyaneum	2.2 ± 0.7 N = 17	289.0 ± 175.4 N = 11	2.1 ± 1.4 N = 18	534.5 ± 151.5 N = 11	2.2 ± 1.4 N = 14	290.9 ± 133.8 N = 14
Fasciolaria t. hollisteri	2.0 ± 0.2 N = 2	3818. 1 N = 1	2.1 ± 0.3 N = 2	2346.9 N = 1	1.2 ± 0.3 N = 2	534.0 N = 1
Fusinus closter	2.3 ± 0.7 N = 5	70.3 ± 6.4 N = 3	4.2 ± 1.5 N = 5	177.7 ± 62.7 N = 3	2.4 ± 1.9 N = 5	182.8 ± 91.3 N = 4
Eualetes tulipa	1.9 ± 0.4 N = 16	0.7 ± 0.2 N = 7	15.1 ± 7.4 N = 13	24.6 ± 20.8 N = 8	7.0 ± 4.7 N = 14	9.8 ± 6.6 N = 6

reported that the vermetid *Petaloconchus montereyensis* Dall, 1919, hatches with a larger shell when it ingests more nurse eggs during intracapsular development. Species used in this study had very similar egg diameters, between 230 and 260 µm, but different proportions of nurse eggs as a source of extraembryonic nutrition. The ratio varied from 1.5 to 446 nurse eggs per embryo. The biochemical analysis of eggs and hatchlings indicated that different families have different balances between initial and final contents of protein, glycogen, and lipid.

The biochemical analysis of the eggs and embryos of the buccinid species indicated that *Buccinum cyaneum* has enough material to account for the developing embryos while *B. undatum* seems to need extra material.

Among the fasciolariids, the biochemical analysis of both species indicated that they do not have enough material in eggs and nurse eggs to account for all of the material in the hatching embryo. These results suggest that there must be other sources of nutrition for the embryos besides nurse eggs, which could be: (1) the intracapsular fluid (De Mahieu *et al.*, 1974, on *Adelomelon brasiliana* Lamarck, 1811; Harasewych, 1978, on *Busycon carica* Gmelin, 1791, and *B. canaliculatum* Linné, 1758; Rivest, 1986, on *Urosalpinx cinerea* Say, 1820; Bayne, 1968, and Stockmann-Bosbach and Althoff, 1989, on *Nucella lapillus* Linné, 1758; Penchaszadeh and Rincón, 1996, on *Prunum prunum* (Gmelin, 1791); and Penchaszadeh, pers. comm., on *Voluta musica* Linné, 1758; (2) the gel matrix that surrounds the embryos (De Mahieu *et al.*, 1974; Hawkins and Hutchinson, 1988); and (3) the egg capsule itself (Bayne, 1968; Hawkins and Hutchinson, 1988; Roller and Stickle, 1988).

The intracapsular fluid of some species has been reported to be rich in proteins and carbohydrates; the contents of these substances decrease through development and are low at the hatching stage (De Mahieu *et al.*, 1974; Harasewych, 1978). The gel matrix of the muricid *Ocenebra erinacea* Linné, 1758, is proteinaceous, presents carbohydrates but lacks lipids (Hawkins and Hutchinson, 1988). These authors suggested that the carbohydrates found in the gel matrix could also represent a pool of non-diffusible molecules as a protection against low salinity conditions as previously discussed by Pechenik (1982, 1983). De Mahieu *et al.* (1974) found that the gel matrix of

Table 4. Total protein, glycogen, and lipid contained in the capsule by the eggs and hatchlings. Values were obtained by multiplying the mean content of protein, glycogen, and lipid of each egg and hatchling by the mean number of eggs and hatchlings per capsule of each species. (Values in mg).

Species	Protein		Glycogen		Lipid		Total	Total
	Eggs	Hatchlings	Eggs	Hatchlings	Eggs	Hatchlings	Eggs	Hatchlings
Buccinum undatum	2.78	1.07	4.98	6.88	1.89	6.25	9.65	14.20
B. cyaneum	1.25	0.87	1.19	1.60	1.26	0.87	3.70	3.34
Fasciolaria t. hollisteri	6.36	26.73	6.58	16.43	3.65	3.74	16.59	46.90
Fusinus closter	0.66	1.19	1.21	3.02	0.71	3.11	2.58	7.32
Eualetes tulipa	0.54	0.13	4.35	4.63	2.03	1.84	6.92	6.60

Table 5. Molecular weight in Kd of the proteins and their percentage of the total electrophoretic bands in egg and hatchling samples. [M = major proteins (> 10% each), m = minor proteins (3-10% each)].

Species	Egg Stage	% of total bands	Hatching Stage	% of total bands
Buccinum undatum	(M) 116, 171, 200	85.3	(M) 30	14.3
	(m) 19, 63	6.1	(m) 31, 37,42, 48,50 52, 56, 62, 65, 82, 97, 126	69.7
B. cyaneum	(M) 114,166	73.4	(M) 29, 49, 61, 206	50.2
	(m) 49, 135	7.9	(m) 36, 43, 52, 63, 71, 81, 98	38.4
Fasciolaria t. hollisteri	(M) 98, 107, 166	79.6	(M) 101, 110, 165	77.7
	(m) 16, 134	9.5	(m) 33, 90	15.6
Fusinus closter	Eggs:		(M) 92, 105	40.0
	(M) 96, 109,164, 247	83.7		
	(m) 211, 263	13.8	(m) 8, 15, 20, 21, 26, 57 63, 174	46.9
	Nurse eggs:			
	(M) 29, 94, 108, 162	56.0		
	(m) 22, 62, 76, 86 133, 145, 204, 239	37.6		
Eualetes tulipa	(M) 60	13.7	(M) 37, 56, 88	34.3
	(m) 51, 56, 64, 81, 89 97, 105, 118,130, 160, 225	80.6	(m) 8, 14, 19, 20, 23, 29 50, 52, 67, 88	57.3

the volutid *Adelomelon brasiliana* maintains a constant proteic composition at all embryonic stages, but its thickness decreases through development. They suggested that a fraction of the proteins in the intracapsular liquid which serves as a source of nutrition for the embryos, originates from the dissolution of this gel matrix.

Studies on the egg capsules of *Ocenebra erinacea* and *Nucella lapillus* (*fide* Hawkins and Hutchinson, 1988, and Bayne, 1968, respectively) indicated that these are mainly composed of glycoproteins. Feeding of the embryos on the egg capsule walls has been hypothesized by Roller and Stickle (1988) for *Thais haemastoma canaliculata* Duclos, 1832. This hypothesis was based on the overall decrease in total capsule dry weight during the intracapsular developmental period as well as the significant decrease in the total organic component of the capsule wall. Miloslavich (in press) found the gel matrix and the egg capsules of *Fasciolaria tulipa hollisteri* and *Fusinus closter* to be rich mainly in glycogen and to a lesser extent in proteins and lipids.

The biochemical analysis of eggs and hatchlings of the vermetid *Eualetes tulipa* indicated that this species has sufficient protein, glycogen, and lipid in eggs and nurse eggs to account for the hatching embryo, and some is left uningested. This positive biochemical balance between eggs and hatchlings is also reflected in the way the embryos ingest nurse eggs. Once the embryos develop the velum, the veligers start to detach yolk particles of the nurse egg mass by means of the velum and feeding on the nurse eggs takes place until the hatching stage (lecithotrophic), when between four and 30 uneaten nurse eggs still remain (Miloslavich and Penchaszadeh, 1992).

Electrophoretic Patterns

There are few electrophoretic works on the eggs and hatchlings of gastropods. Morrill (1973) analyzed the phosphatase activity of the developing embryo of the pulmonate *Physa fontinalis* Linné, 1758, and found at least ten distinct phosphatase bands. Sullivan and Bonar (1984) reported over 100 bands on one-dimensional electrophoretic gels of the embryo of *Ilyanassa obsoleta* Say, 1822, with very variable molecular weights. The latter is a much higher number than the number of bands reported for any species in the present work.

The electrophoretic gel showed that different families have different patterns of egg proteins. Within the buccinids (both *Buccinum*), the patterns of egg proteins of the two species were very similar. The fasciolariid species (*Fasciolaria* and *Fusinus*) had two similar proteins of

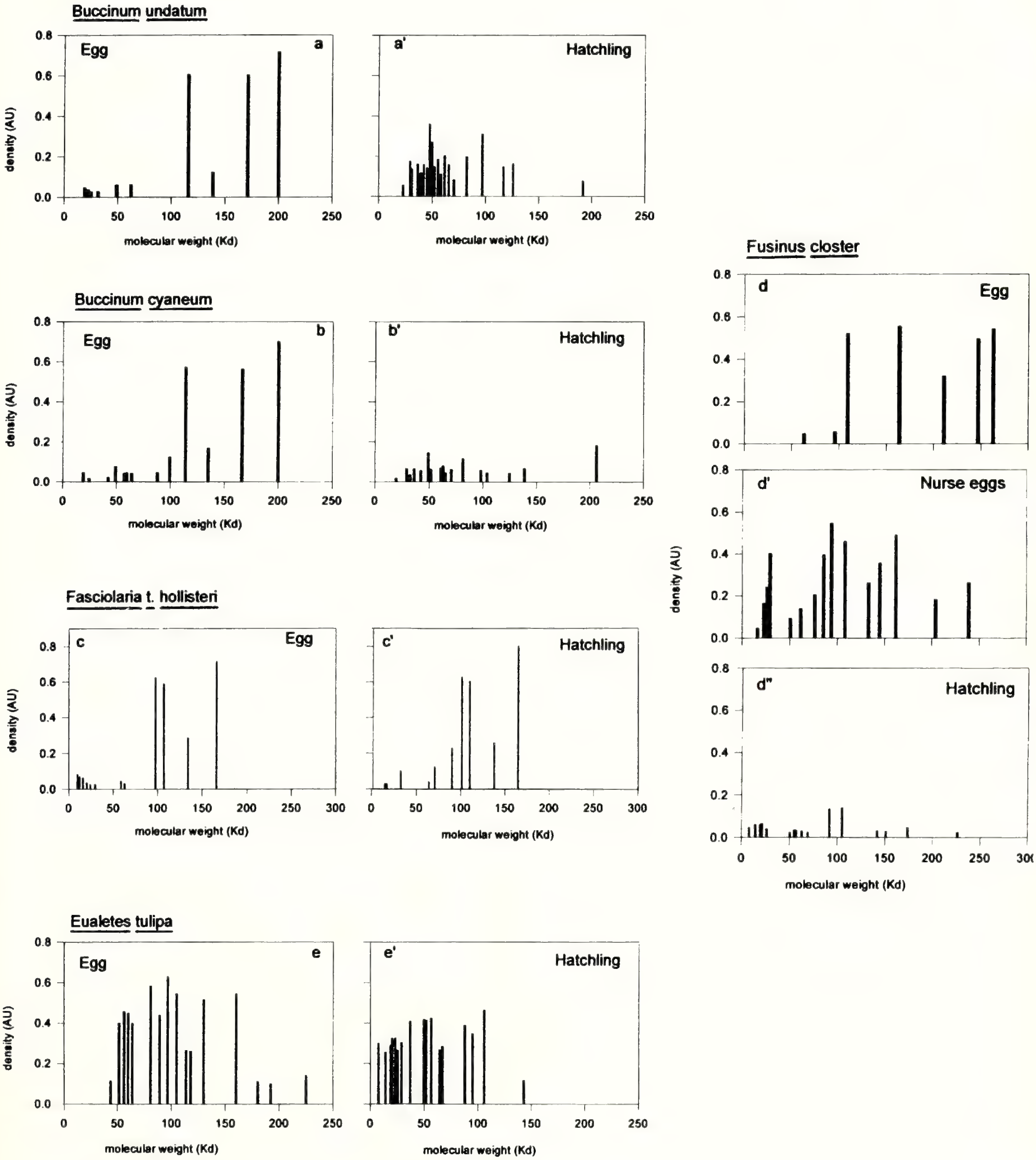

Fig. 2. Density readings in absorbance units (AU) of the electrophoretic gels of eggs and hatchlings in Fig. 1. Each bar represents a protein, the molecular weight indicated in Kd.

around 100 and 160 Kd, with *Fusinus closter* having additional higher molecular weight proteins. The nurse eggs of *F. closter,* which disintegrate when the developing embryos are at a very early stage (8-16 cell stage), presented three different proteins from those of the eggs (both developing and non-developing) at the initial stage. This means that the disintegrated nurse egg material contains three proteins (MW around 29.2, 76.3, and 85.5 Kd) that were not present prior to the disintegration. These proteins could be either synthethized at disintegration or could be the result of the hydrolysis of the higher molecular weight proteins into smaller fragments. The first hypothesis remains to be proven, while the second one could be verified by comparing the density readings of the bands at the egg stage with those of the disintegrated material. If the second hypothesis is true, there should be a decrease in the density of the HMW bands of the disintegrated material in comparison to the egg stage because that part of it would generate the new bands. This decrease in density does not occur, so the question of the origin of the new bands remains. The pattern of egg proteins of the vermetid *Eualetes tulipa* is characterized by several more LMW proteins than any other species considered in this study. An interesting fact is that the eggs of the five species shared a major protein of around 160-170 Kd (minor for *E. tulipa*); this protein is most probably an egg reserve protein for the embryo, which would be interesting to characterize and verify its universality among other prosobranchs.

The comparison of the egg protein pattern with the hatchling protein pattern indicates that there are taxonomic differences at the family level. The buccinids convert most of the egg proteins to different LMW proteins. The fasciolariids also convert most of the HMW proteins to LMW proteins, but *Fasciolaria tulipa hollisteri* retains most of the egg HMW proteins at hatching. This retention and the three-fold increase in the final biochemical content from egg to hatching suggests that *F. t. hollisteri* uses even more protein from other sources in the capsule than mentioned before. The vermetid *Eualetes tulipa* converts the HMW proteins of the egg to LMW at the hatching stage. However, several of these HMW proteins are still present in the hatchling, with the bands of about the same density as the egg stage. This fact is most probably due to the way in which this species ingests the nurse eggs as discussed previously.

The resorption of nurse eggs by the embryo depends on the type of adelphophagy and has been explained for several species by Fioroni and Sandmeier (1964), Fioroni (1967, 1977), and Fioroni and Schmekel (1976). In the case of *Buccinum undatum, B. cyaneum,* and *Fasciolaria tulipa hollisteri* which ingest whole nurse eggs at an early stage (Martel *et al.*, 1986; Miloslavich and Dufresne, 1994; Penchaszadeh and Paredes, in press), these are swallowed by means of the esophagus and the food stored in special entodermal nutritive sacs (intra- or extracellular). In *Eualetes tulipa*, a species that mechanically breaks nurse eggs into small pieces prior to ingestion (Miloslavich and Penchaszadeh, 1992), feeding on nurse eggs is continuous through development. In *Fusinus closter*, even when the nurse eggs are broken down into small fragments (the process causing disintegration has not been studied), there is a well-defined early ingestion stage (Miloslavich and Penchaszadeh, in press).

The fasciolariid and vermetid species retained more nurse egg proteins at the hatching stage. This is most significant in *Fasciolaria tulipa hollisteri.* In this species, the ratio of nurse eggs to developing embryos was highest of all species studied herein and it also showed the greatest increase in protein content. Each embryo swallowed 400 nurse eggs and the protein content of the embryo increased 2000-fold from egg to hatching. This agrees with Fioroni (1982, 1988) who suggested that the nurse egg reserves present in the hatchling seem to be related to the amount of extraembryonic nutrition.

Despite the 100-fold increase in protein content from egg to hatching and the fact that there are around 100 nurse eggs for each developing embryo, the content of nurse egg proteins of the hatchlings of both *Buccinum* species were low compared to the fasciolariid and vermetid species. This is evident when compared to *Fasciolaria tulipa hollisteri* which also has intracapsular metamorphic development and the same mode of adelphophagy. However, the *Buccinum* species have longer developmental periods (5 mo for *B. undatum* and 9 mo for *B. cyaneum*) compared to *F. t. hollisteri* (*ca.* 3 mo), *Fusinus closter* (6 to 7 wk) and *Eualetes tulipa* (21 days). It is most probable that during such a long period of time, the nurse egg proteins are consumed in a different way or rate than in species with shorter developmental periods.

From this study, it can be concluded that taxonomic differences at the family level are expressed in the biochemical balance of eggs and hatchlings, in the electrophoretic pattern of egg proteins, and in the conversion of the egg proteins into other proteins at the hatching stage. Given the negative balance between the initial and final stages in fasciolariids, the possibility of other extraembryonic sources of food in the capsule must be investigated.

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LITERATURE CITED

Amio, M. 1963. A comparative embryology of marine gastropods, with ecological considerations. *Journal of the Shimonoseki University of Fisheries* 12(2,3):229-357.

Bandel, K. 1975. Embryonalgehause karibischer Meso- und Neogastropoden (Mollusca). *Akademie der Wissensschaften und der Literatur, Mainz* 1:1-133.

Bandel, K. 1976. Morphologie der Gelege und okologische Beobachtungen an Buccinaceen (Gastropoda) aus der sudlichen Karibischen See. *Bonn Zoologische Beitrabe* 1/2:98-132.

Bayne, C. J. 1968. Histochemical studies on the egg capsules of eight gastropod molluscs. *Proceedings of the Malacological Society of London* 38:199-212.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* 72(1/2):248-254.

D'Asaro, C. N. 1970. Egg capsules of some prosobranchs from the Pacific coast of Panama. *The Veliger* 13(1):37-43.

D'Asaro, C. N. 1986. Egg capsules of eleven marine prosobranchs from northwest Florida. *Bulletin of Marine Science* 39(1):76-91.

De Mahieu, G., P. E. Penchaszadeh, and A. B. Casal. 1974. Algunos aspectos de las variaciones de proteínas y aminoácidos libres totales del líquido intracapsular en relación al desarrollo embrionario en *Adelomelon brasiliana* (Lamarck, 1811) (Gastropoda, Prosobranchia, Volutidae). *Cahiers de Biologie Marine* 25:215-227.

Fioroni, P. 1966. Zur Morphologie und Embryogenese des Darmtraktes und der transitorischen Organe bei Prosobranchiern (Mollusca, Gastropoda). *Revue Suisse de Zoologie* 73:45-876.

Fioroni, P. 1967. Quelques aspects de l'embryogenèse des Prosobranches (Mollusca, Gastropoda). *Vie et Milieu, Serie A. Biologie Marine* 18(1A):153-174.

Fioroni, P. 1977. Problems and results in developmental biology of molluscs. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 1977:216-228.

Fioroni, P. 1982. Entwicklungstypen und Schlupfstadien bei Mollusken-Einige allgemeine Befunde. *Malacologia* 22(1-2):601-609.

Fioroni, P. 1988. Die Prosobranchier-Entwicklung mit Nahreiern. *Zoologischer Anzeiger* 221:201-247.

Fioroni, P. and A. Portmann. 1968. Zur Morphogenese der Verdauunsorgane und der Larvalorgane von *Fusus* (Gastropoda, Prosobranchia). *Revue Suisse de Zoologie* 75(46):833-882.

Fioroni, P. and E. Sandmeier. 1964. Ueber eine neue Art der Naehreierbewaeltigung bei Prosobranchierveligern. *Vie et Milieu* Suppl. 17:235-249.

Fioroni, P. and L. Schmekel. 1976. Ontogeneses of gastropods with rich nutritive resources. *Zoologische Jahrbucher (Anatomie)* 96:74-171.

Fretter, V. and A. Graham. 1962. *British Prosobranch Molluscs. Their Functional Anatomy and Ecology.* Ray Society, London. 755 pp.

Glaser, O. C. 1905. Uber den kannibalismus bei *Fasciolaria tulipa* (var. *distans*) und deren larvale Excretionsorgane. *Zeitschrift für wissenschaftliche Zoologie* 80:80-121.

Gohar, H. A. F. and M. Eisawy. 1967. The egg masses and development of five rachiglossan prosobranchs from the Red Sea. *Publication of the Marine Biological Station Al-Ghardaqa* 14:215-268.

Gosselin, L. A. and F.-S. Chia. 1994. Feeding habits of newly hatched juveniles of an intertidal predatory gastropod *Nucella emarginata* (Deshayes). *Journal of Experimental Marine Biology and Ecology* 176 (1):1-14.

Hadfield, M. G. 1970. Observations on the anatomy and biology of two California vermetid gastropods. *The Veliger* 12(3):301-309.

Hadfield, M. G. 1989. Latitudinal effects on juvenile size and fecundity in *Petaloconchus* (Gastropoda). *Bulletin of Marine Science* 45(2):369-376.

Hadfield, M. G. and D. K. Iaea. 1989. Velum of encapsulated veligers of *Petaloconchus* (Gastropoda), and the problem of re-evolution of planktotrophic larvae. *Bulletin of Marine Science* 45(2):377-386.

Hadfield, M. G., E. A. Kay, M. U. Gillette, and M. C. Lloyd. 1972. The Vermetidae (Mollusca: Gastropoda) of the Hawaiian Islands. *Marine Biology* 12:81-98.

Harasewych, M. G. 1978. *Biochemical Studies of the Hatching Process in* Busycon. Masters Thesis, University of Delaware, Newark. 52 pp.

Hawkins, L. E. and S. Hutchinson. 1988. Egg capsule structure and mechanism of *Ocenebra erinacea* L. (Prosobranchia: Muricidae). *Journal of Experimental Marine Biology and Ecology* 119:269-283.

Hughes, R. N. 1978. The biology of *Dendropoma corallinaceum* and *Serpulorbis natalensis*, two South African vermetid gastropods. *Zoological Journal of the Linnean Society* 64:111-127.

Hyman, O. W. 1923. Spermic dimorphism in *Fasciolaria tulipa. Journal of Morphology* 37(1):307-383.

Hyman, O. W. 1935. Natural partial fertilization in *Fasciolaria tulipa. Journal of Morphology* 41:267-281.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 227:680-685.

Martel, A., D. H. Larrivée, and J. H. Himmelman. 1986. Behaviour and timing of copulation and egg laying in the neogastropod *Buccinum undatum* L. *Journal of Experimental Marine Biology and Ecology* 96:27-42.

Miloslavich, P. In press. Biochemical composition of prosobranch egg capsules. *Journal of Molluscan Studies.*

Miloslavich, P. and L. Dufresne. 1994. Development and effect of female size on egg and juvenile production in the neogastropod *Buccinum cyaneum* from the Saguenay Fjord. *Canadian Journal of Fisheries and Aquatic Sciences* 51:2866-2872.

Miloslavich, P. A. and P. E. Penchaszadeh. 1992. Reproductive biology of *Vermetus* sp. and *Dendropoma corrodens* (Orbigny, 1842): two vermetid gastropods from the southern Caribbean. *The Veliger* 35(1):78-88.

Miloslavich, P. and P. Penchaszadeh. 1994. Spawn and development of *Engoniophos unicinctus* Say, 1825 (Gastropoda, Prosobranchia) from the southern Caribbean. *The Veliger* 37(4):425-429.

Miloslavich, P. and P. Penchaszadeh. In press. Spawn and development of *Fusinus closter* Philippi 1850 (Gastropoda, Prosobranchia) from the Venezuelan Caribbean. *The Veliger.*

Morrill, J. B. 1973. Biochemical and electrophoretic analysis of acid and alkaline phophatase activity in the developing embryo of *Physa fontinalis* (Gastropoda, Pulmonata). *Acta Embryologiae et*

Morphologiae Experimentalis 1973:61-82.

Pechenik, J. A. 1982. Ability of some gastropod egg capsules to protect against low salinity stress. *Journal of Experimental Marine Biology and Ecology* 63:195-208.

Pechenik, J. A. 1983. Egg capsules of *Nucella lapillus* (L.) protect against low salinity stress. *Journal of Experimental Marine Biology and Ecology* 71:165-179.

Pechenik, J. A. 1986. The encapsulation of eggs and embryos by molluscs: an overview. *American Malacological Bulletin* 4:165-172.

Penchaszadeh, P. E. 1971a. Observaciones sobre la reproducción y ecología de *Dorsanum moniliferum* (Valenciennes, 1834) (Gastropoda, Buccinidae) en la región de Mar del Plata. *Neotropica* 17(53):49-54.

Penchaszadeh, P. E. 1971b. Aspectos de la embriogénesis de algunos gasterópodos del género *Buccinanops* d'Orbigny, 1841 (Gastropoda, Prosobranchiata, Buccinidae). *Physis* 30(81):474-482.

Penchaszadeh, P. E. 1973. Nuevas observaciones sobre la reproducción de *Buccinanops gradatum* (Deshayes, 1884) (Gastropoda, Prosobranchiata, Dorsaninae). *Physis* 32(84):15-18.

Penchaszadeh, P. E. and C. Paredes. In press. Reproduction of *Fasciolaria tulipa hollisteri* Weisbord 1962 (Gastropoda: Prosobranchia) from the southern Caribbean. *Malacological Review*.

Penchaszadeh, P. and A. Rincón. 1996. Egg capsules and development of *Prunum prunum* (Gmelin, 1791) (Prosobranchia: Marginellidae) from the Venezuelan Caribbean. *The Veliger* 39(1):83-86.

Portmann, A. 1925. Der Einfluss der Nähreier auf die Larven-entwicklung von *Buccinum* und *Purpura*. *Zeitschrift fur Morphologie und Okologie der Tiere* 3:526-541.

Rivest, B. R. 1986. Extra embryonic nutrition in the prosobranch gastropod *Urosalpinx cinerea* (Say, 1822). *Bulletin of Marine Science* 39(2):498-505.

Roller, R. A. and W. B. Stickle. 1988. Intracapsular development of *Thais haemastoma canaliculata* (Gray) (Prosobranchia: Muricidae) under laboratory conditions. *American Malacological Bulletin* 6(2):189-197.

Shuto, T. 1974. Larval ecology of prosobranch gastropods and its bearing on biogeography and paleontology. *Lethaia* 7:239-256.

Stockmann-Bosbach, R. and J. Althoff. 1989. A correlated morphological and biochemical study of the intracapsular fluid of *Nucella lapillus* (Gastropoda, Prosobranchia, Muricidae). *Marine Biology* 102:283-289.

Sullivan, C. H. and D. B. Bonar. 1984. Biochemical characterization of the hatching process of *Ilyanassa obsoleta*. *Journal of Experimental Zoology* 229(2):223-234.

Thorson, G. 1935. Studies on the egg-capsules and development of Arctic marine prosobranchs. *Meddelser om Gronland* 100:1-71.

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A new land snail of the genus *Gastrocopta* from Nicaragua (Pulmonata: Vertiginidae), and its relationship to species from northeastern South America

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Abstract: *Gastrocopta (Immersidens) gularis,* sp. nov., is described from the submesic Pacific coastal region of western Nicaragua and Costa Rica. It is closely related to species described from regions of similar habitats in northern South America.

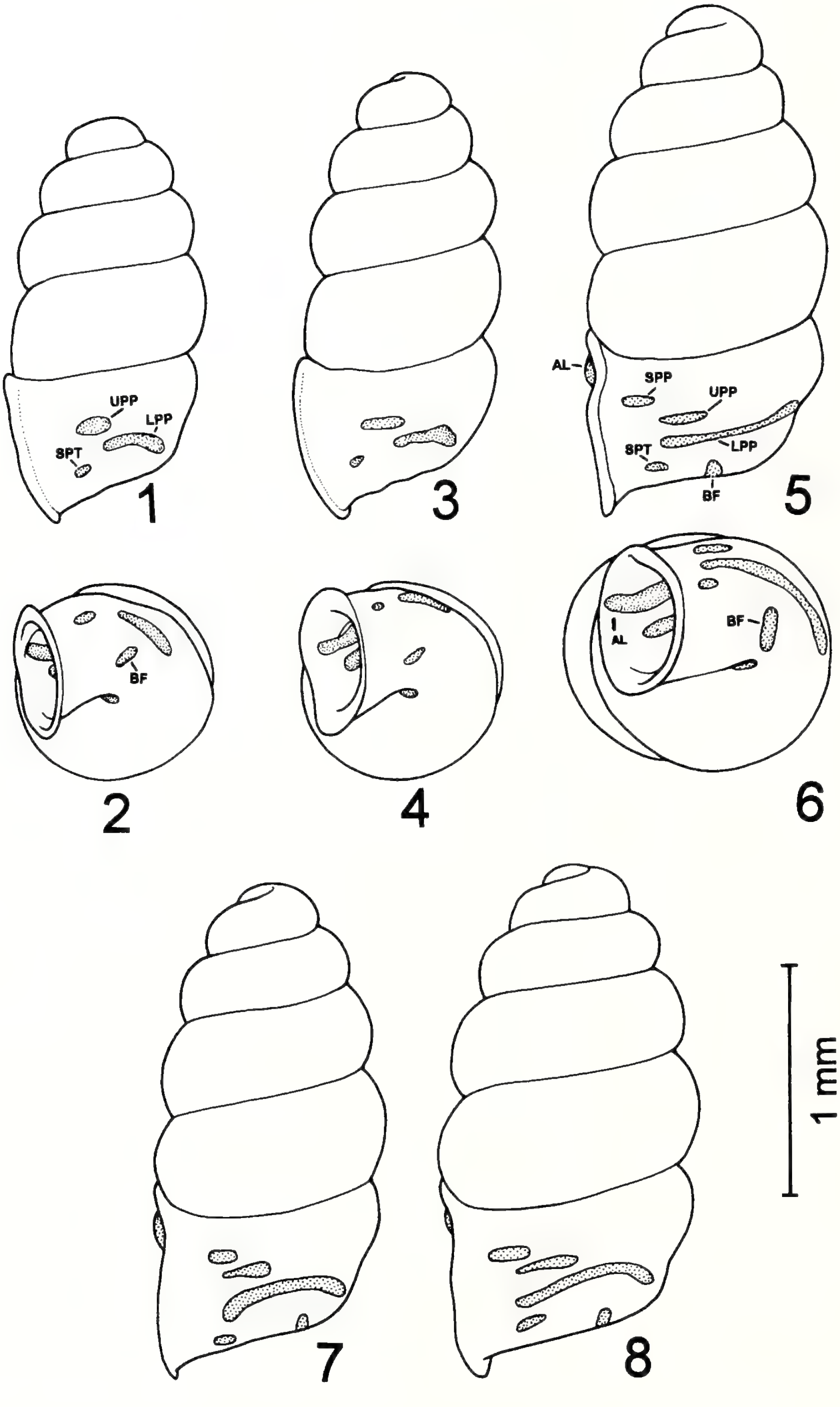
Key words: terrestrial Gastropoda, Vertiginidae, *Gastrocopta,* Nicaragua, Pulmonata, *Immersidens*

The vertiginid landsnail fauna of Central America is not well documented. Those species that have been reported from the region belong to species groups that are widespread in North America and the West Indies. The new *Gastrocopta* described herein is of particular interest because it belongs to a species group otherwise found in lowland submesic habitats in northeastern South America. Acronyms used for museum specimens cited in this paper are explained under Acknowledgments.

Gastrocopta (Immersidens) gularis, sp. nov.
(Figs. 5-8, 15-19, 21, 25)

Diagnosis. A species of the subgenus *Immersidens* Pilsbry and Vanatta, 1900, by virtue of having the parietal and angular lamellae unite internally to form a configuration resembling the Greek letter lambda (λ) in shape, the basal fold is transverse to the opening, and the palatal plicae are not born on a callous ridge. It is most closely related to species from northern South America because of the absence of an exterior ridge parallel to the peristome, and the heavy development of the angular-parietal lamella. It is most similar to *Gastrocopta colombiana* Pilsbry, 1921, from which it differs by its larger, stockier shell, its slightly lower whorl-count, its stronger aperture dentition, the straight anterior half of the lower palatal plica which is not strongly arched toward the base of the whorl, and the more posteriorly located basal fold.

Description. Periostracum light brown. Aperture white. Shell elongate-tapered in shape, about 0.46-0.52 times as wide as long. Aperture height about 0.36-0.40 times the length of the shell. Spire obtusely rounded at apex. Last whorl slightly wider than penultimate whorl; without an external callus or ridge parallel to peristome; with a very shallow external longitudinal impression about 1/4 whorl long, parallel to and overlying the larger of the palatal folds. Mature shell with 4.8-5.3 evenly rounded whorls that are separated by a deep suture. Columellar wall of last half-whorl vertically flattened and nearly straight longitudinally. Umbilicus with a small, narrow, elliptical perforation that is sufficiently wide to permit visibility of the previous whorl. Shell nearly smooth, sculptured with weak, irregular, incremental growth threads that are most developed along the suture. Aperture trapezoidal in shape, about 0.77-0.87 times as wide as high; vertical in lateral profile. Peristome thin, complete across parietal wall where it is narrowly attached to preceding whorl; broadly expanded and reflected; narrowest at palatal-parietal corner. Internal barrier consisting of seven deeply immersed teeth. An additional tubercle can be present in some specimens. The parietal and angular lamellae are strong and parallel with a deep trough between them. Their bases form a coalesced buttress, and they boldly protrude basely half-way across the internal opening. The angular lamella projects slightly ahead of the peristome in lateral profile (Figs. 5, 7-8, 16-17, 25) where it flexes toward the right. It extends almost from the margin of the peristome to deep within the aperture, and it is highest just before it unites with the parietal lamella. The parietal lamella begins deeper within the aperture. It is low anteriorly and is highest posterior to where the angular lamella joins it. Its inner end curves



Figs. 1-8. Outline drawings of *Gastrocopta* showing the positions of the internal lamellae and plicae. **Figs. 1-4.** *G. hummelincki* (UF 246549). **Figs. 5-8.** *G. gularis*, sp. nov.; Figs. 5-6, holotype (UF 247775); Figs. 7-8, paratypes (UF 247776); note the relatively obese shape and the forward projection of the angular lamella in Figs. 5, 7-8. (AL, angular lamella; BF, basal fold; LPP, lower palatal plica; SPP, superior palatal plica; SPT, subpalatal plica; UPP, upper palatal plica).

strongly toward the outer wall. The columellar lamella is equally strong and extends outward almost to the parietal lamella. Deep within the aperture and at the edge of the columella it makes a right-angle bend toward the base of the shell. The basal fold lies deep within the aperture. It is high, flat-topped, and radial in alignment, transverse to the aperture. The palatal wall bears three parallel plicae (Figs. 5, 7-8): the suprapalatal, upper palatal, and lower palatal. The suprapalatal plica is small, tubercular, and lies just within the aperture. The upper palatal plica is more elongate and lies below and slightly receded to the suprapalatal plica. The lower palatal plica lies on the middle of the palatal wall and underlies the external furrow. It is straight or slightly gibbous, highest at its anterior end, and extends internally for about 1/4 of a whorl where it is slightly enlarged again. A small subpalatal tubercle is usually present anterior to the lower plica and slightly below it, as in the holotype. Shell measurements are given in Table 1.

Type material and locality. Nicaragua, Dept. Managua, Lago de Xiloa (12°14' N, 86°20' W). Holotype: UF 247775. Paratypes: UF 247776 (18); UCA 94:54 (42), same data as the holotype. The holotype of *Gastrocopta gularis* is illustrated in outline drawings to show the shape of the shell and the relative positions of the apertural lamellae (Figs. 5-6). It is not illustrated with SEM micrographs because to do so would require gold plating of the specimen.

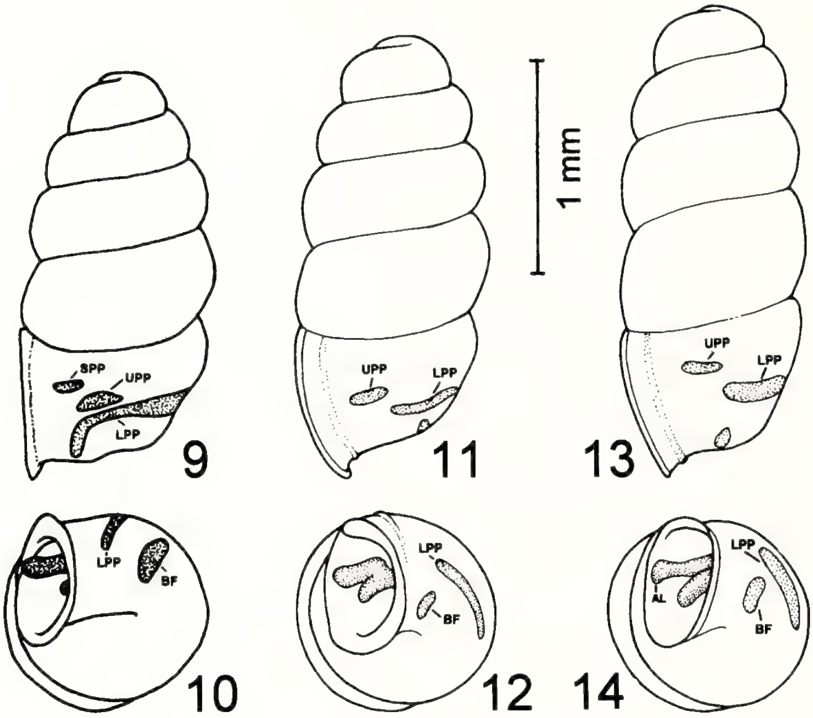
Lago de Xiloa is an irregularly circular lake of volcanic origin approximately 2.5 km in diameter. It is on the Chiltepe Peninsula, 12 km northwest of Managua City and along the southwestern side of Lago de Managua. Lago de Xiloa was once inundated as part of Lago de Managua, but it became isolated as water levels subsided. The two lakes are now separated by a distance of 1 km. The type locality is at the western end of Lago de Xiloa in an area partly covered with a patch of submesic tropical savanna. Brush-fires occasionally scar the area. Human activity is reduced to transient hunters and fishermen. *Gastrocopta gularis* was found under leaf and rock cover in a mixture of soil and pumice gravel characteristic of the lake shore. All specimens were collected close to the water at the lower part of the sloping shore of the lake.

Other gastropods found at the type locality were: *Gastrocopta servilis servilis* (Gould, 1843), *G. pellucida hordeacella* (Pilsbry and Vanatta, 1900), *G. gemnidens* Pilsbry, 1917, *Pupisoma dioscoricola* (C. B. Adams, 1845), *P.* sp., *Bothriopupa tenuidens* (C. B. Adams, 1841), *Beckianum beckianum* (Pfeiffer, 1846), *B. sinistrum* (Martens, 1898), *Lamellaxis gracile* (Hutton, 1834), *L. micra* (Orbigny, 1835), *Subulina octona* (Bruguière, 1792), *Opeas pumilum* (Pfeiffer, 1822), *Leptinaria* sp., *Cecilioides consobrinus veracruzensis* Crosse and Fischer, 1877, *C. aperta* (Swainson, 1840), *Euglandina cumingi* (Beck, 1837), *Miradiscops* sp., *Orthalicus ferussaci* (Martens, 1863), *Bulimulus corneus* (Sowerby, 1833), *Drymaeus multilineatus* (Say, 1925), *Succinea* cf. *guatemalensis* Morelet, 1849, *Guppya gundlachi* (Pfeiffer, 1839), *Hawaiia minuscula* (Binney, 1840), *Glyphyalinia paucilirata* (Morelet, 1841), *Praticolella griseola* (Pfeiffer, 1841), *Thysanophora caecoides* (Tate, 1870), and *T.* sp. These species are common in the submesic Pacific zone of Nicaragua. Specimens of all of these species are deposited in both the Universidad de América Central and the Florida Museum of Natural History.

Distribution. Submesic regions of southwestern Nicaragua and northwestern Costa Rica. COSTA RICA.- Prov. Guanacaste: Area de Conservación Guanacaste, Parque Nacional Santa Rosa, Sector Argelia, banks of the Río Nisperal, 85°39'25" N, 10°48'33" W, 50 m alt. (INBIO 1480405); Area de Conservación Guanacaste, Parque Nacional Santa Rosa, 2.5 km N Estación Argelia, 10°47'50" N, 85°39'15" W, 20 m alt. (INBIO 1480357), Area de Conservación Tempisque, Parque Nacional Palo Verde, Sector Palo Verde, Sendero Guayacán, 10°21'02" N, 85°21'08" W, 50 m alt. (INBIO 1474875, UF 244467). NICARAGUA.- Dept. Boaco: Las Canoas, 12°31'36" N, 85°52'50" W (UCA 92:93). Dept. León: Lago Asososca, 12°26' N, 86°40' W (UCA 94:47). Dept. Managua: Platanal, 12°27'15" N, 86°05'34" W (UCA 95:89); Tamarindo, 12°29'30" N, 86°05'25" W (UCA 95:57). Dept. Masaya: Lago Apoyo, 11°55' N, 86°03' W (UCA 93:87). Dept. Matagalpa: Dario, 12°43' N, 86°12' W (UCA 93:46A; UF 247777).

Remarks. *Gastrocopta gularis* belongs to the subgenus *Immersidens* Pilsbry and Vanatta, 1900. Various species of Immersidens occur in South America. Other species occur in the southwestern United States, Mexico, and Guatemala. The subgenus is characterized by having the parietal and angular lamellae unite internally to form a configuration resembling the Greek letter lambda (λ) in shape. The basal fold is transverse to the opening, and the palatal plicae are not borne on a callous ridge.

Gastrocopta gularis is similar to *G. colombiana* Pilsbry, 1921, *G. humellincki* Haas, 1960, and *G. iheringi* (Suter, 1900), which are South American species. They are alike in shape, size, number of whorls, the lack of an external crest behind the peristome, the brusque aperture dentition, the parallel alignment and heavy structure of the parietal-angular lamella complex, and the shape of the columellar lamella. They differ from each other by aspects of shell shape and aperture dentition as discussed below. They contrast with North American species of *Immersidens* by the heavy construction and parallel alignment of the parietal-angular lamella, as opposed to the weaker construction and the more open λ-shaped configuration of the parietal-angular lamellae. The heavy downward-turned columellar



Figs. 9-14. Outline drawings of *Gastrocopta* showing the positions of the internal lamellae and plicae. **Figs. 9-10.** *G. colombiana* (FMNH 65394). **Figs. 11-12.** *G. d. dalliana* (SBMNH 71098). **Figs. 13-14.** *G. d. bilamellata* (SBMNH 71213). (AL, angular lamella; BF, basal fold; LPP, lower palatal plica; SPP, superior palatal plica; UPP, upper palatal plica).

lamella is also unique, except for the appearance of a similar but weaker development of this trait in the North American *G. dalliana bilamellata* (Sterki and Clapp, 1909), which occurs in Arizona, Chihuahua, and Sonora (Bequaert and Miller, 1973; Naranjo-Garcia, 1991) (Figs. 13-14).

Gastrocopta colombiana (Figs. 9-10; Table 2) is found along the Caribbean coastal region of Colombia. It was described from Puerto Colombia, Dept. Atlántico, Colombia (Pilsbry, 1921). It is a slightly smaller and more slender species than *G. gularis*. It has a slightly higher whorl-count. Its aperture dentition is similar to that of *G. gularis,* but weaker. Also, the anterior half of the lower palatal plica is strongly arched toward the base of the whorl so that its forward end lies almost transverse to the base of the whorl. The basal fold is more anteriorly located than it is in *G. gularis.*

Gastrocopta hummelincki is from Margarita Island, Venezuela. In *G. hummelincki,* the angular lamella does not extend to the edge of the parietal lip. A suprapalatal plica is usually absent (Figs. 1, 3). When present, it is a low narrow tubercle just inside the peristome (Figs. 21-22). The species has a single, short, deeply immersed upper palatal plica (Figs. 1, 3). The lower palatal plica is relatively short, deeply immersed within the aperture, and is weakly arched toward the base. The subpalatal plica is short and compressed-tubercular. It is nearly aligned with the lower

Table 1. *Gastrocopta gularis*, sp. nov. Measurements (in mm) based on 19 specimens. Measurements are converted to mm from ocular micrometer units. Ratios are based on micrometer units. (ApH, aperture height; ApW, aperture width; SD, standard deviation, SH, shell height; SW, shell width).

	SH	SW	ApH	ApW	whorls	SW/SH	ApH/SH	APW/ApH
Holotype	2.31	1.09	0.83	0.69	5.3	0.47	0.40	0.83
Paratypes (N = 18)								
min.	2.11	1.06	0.76	0.66	4.70	0.46	0.35	0.77
max.	2.57	1.22	0.92	0.76	5.30	0.52	0.40	0.87
Mean	2.27	1.10	0.83	0.70	5.09	0.49	0.37	0.84
SD	0.13	0.05	0.04	0.03	0.19	0.02	0.03	0.04

Table 2. *Gastrocopta columbiana* Pilsbry, 1921. Measurements (in mm) based on 11 specimens (FMNH 65394). Measurements are converted to mm from ocular micrometer units. Ratios are based on micrometer units. (ApH, aperture height; ApW, aperture width; SD, standard deviation, SH, shell height; SW, shell width).

	SH	SW	ApH	ApW	whorls	SW/SH	ApH/SH	APW/ApH
min.	1.91	0.96	0.73	0.66	5.20	0.45	0.33	0.80
max.	2.27	1.02	0.83	0.73	5.60	0.52	0.96	0.96
Mean	2.10	1.00	0.78	0.69	5.35	0.48	0.37	0.89
SD	2.27	1.10	0.83	0.70	5.09	0.49	0.37	0.84

palatal plica from which it is separated by a gap (Figs. 1, 3). The short tubercular basal fold of *G. hummelincki* is oblique in orientation (Figs. 2, 4, 21-22), and not transverse as in other species of *Immersidens*. Shell measurements are given in Table 3.

Haas (1960) incompletely described *Gastrocopta hummelincki*, and his illustrations do not show sufficient detail for critical comparisons. He correctly identified the upper palatal plica, but he failed to describe its length or its location relative to its distance from the lip. The structure referred to as the basal fold is the lower palatal plica. The structure Haas called the lower palatal plica is actually the transverse basal fold.

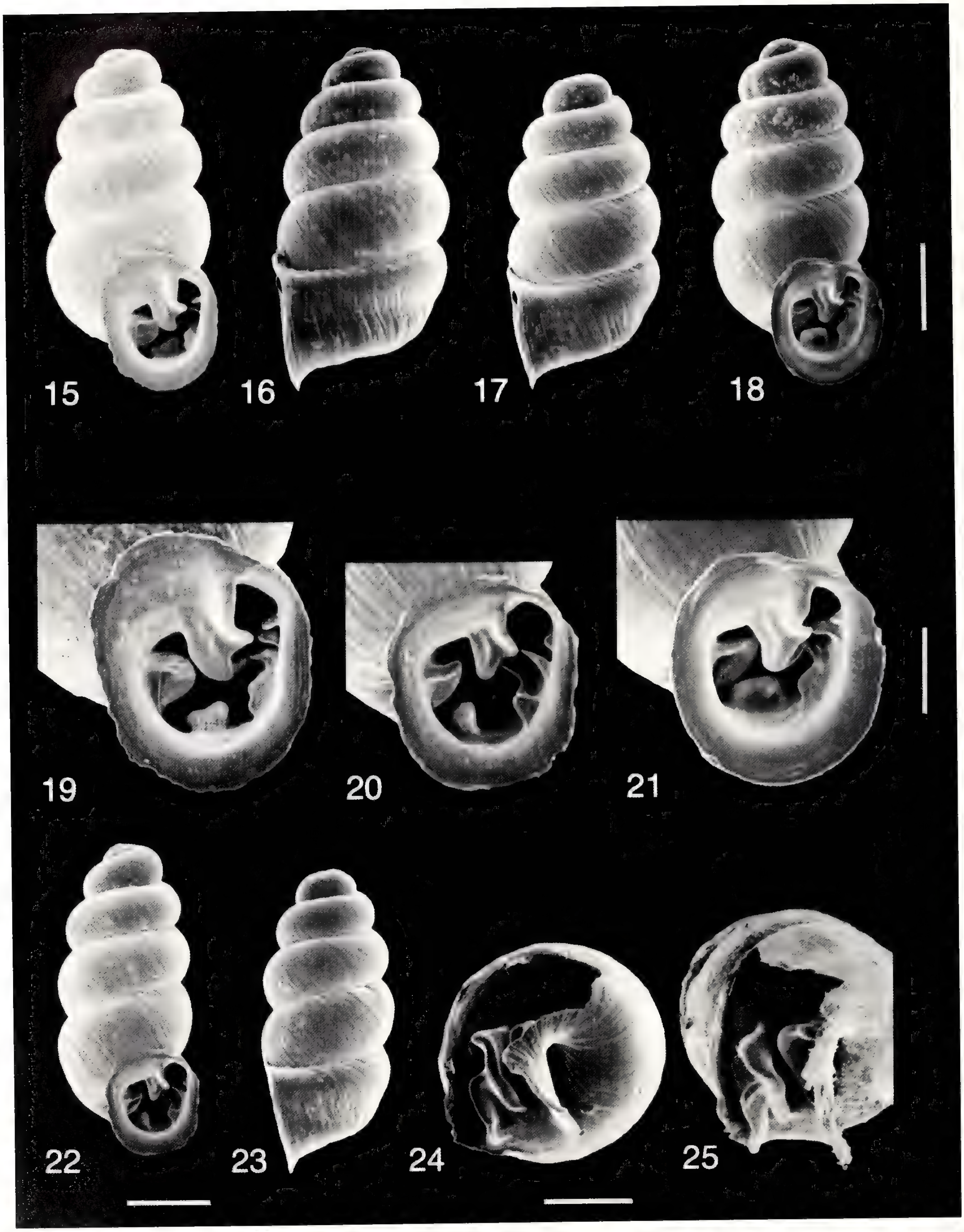
Pilsbry (1916: 101-102, pl. 17, fig. 16) provided an excellent description and illustration of *Gastrocopta iheringi*. This species has a rimate umbilical perforation that occludes visibility of the previous whorl. The aperture is elongate-oval in shape. The peristome is narrow and is discontinuous across the parietal wall in adult specimens. The parietal lamella is highest where it is joined by angular lamella, and is aligned between the upper and the lower palatal plicae. The upper and lower palatal plicae are short and lamellar. The upper palatal plica is posterior to the lower palatal plica. The lower palatal plica is short and is compressed-tubercular in shape. The basal fold is about twice as long as in *G. gularis* and lies transverse to the aperture.

Gastrocopta iheringi occurs in southeastern Brazil (Suter, 1900). Other country records are doubtful. Richards and Hummelinck (1940: 11) reported *G. iheringi* from several Caribbean islands off the Venezuelan coast, including Margarita Island, as well as several localities from the mainland. These same specimens were reported upon by Haas (1960). Specimens recorded by Richardson and Hummelinck from Margarita Island are *G. hummelincki* (see Haas, 1960: 16). I have examined the specimens that they and Haas recorded from Dept. La Guajira, Colombia (FMNH 65273, FMNH 65393, FMNH 65394). They are *G. colombiana*. Specimens recorded by these authors from Isla Chacachacare, Trinidad (FMNH 65389) apparently are an undescribed species of *Gastrocopta*. Arias (1955: 144-145) also recorded *G. iheringi* from Venezuela, following the earlier report by Richards and Hummelinck. Arias quoted Pilsbry's description of *G. iheringi*, but he did not describe or illustrate any of his specimens from Venezuela. Critical examination of these specimens is necessary to confirm the occurrence of *G. iheringi* in Venezuela. However, its occurrence there is doubtful.

Gastrocopta dalliana bilamellata from Arizona and Sonora is similar to *G. gularis* in having the posterior edge of the columellar lamella curved downward. The columellar lamella is horizontal in *G. d. dalliana* (Sterki, 1898). Aside from the configuration of the columellar lamella in *G. d. bilammelata, G. dalliana* and its subspecies have little in common morphologically with *G. gularis.* Their shells are nearly cylindrical, they have an external crest behind the peristome, their aperture dentition is much weaker, their parietal-angular lamellae are broadly λ-shaped, and their lower palatal plica is short and deeply immersed (Figs. 13-14). Similarities between *G. d. bilamellata* and *G. gularis* in the curvature of the columellar lamella must be considered convergent. Other characters, including the external post-labial crest in *G. d. bilamellata* indicates a closer relationship of that species to other North American

Table 3. *Gastrocopta hummelincki* Haas, 1960. Measurements (in mm) based on 10 specimens (UF 246549). Measurements are converted to mm from ocular micrometer units. Ratios are based on micrometer units. (ApH, aperture height; ApW, aperture width; SD, standard deviation, SH, shell height; SW, shell width).

	SH	SW	ApH	ApW	whorls	SW/SH	ApH/SH	APW/ApH
min.	1.78	1.83	0.59	0.53	4.50	0.46	0.32	0.80
max.	2.01	2.01	0.73	0.63	4.90	0.49	0.37	0.99
Mean	1.92	0.91	0.67	0.59	4.76	0.47	0.35	0.89
SD	0.07	0.04	0.03	0.03	0.13	0.01	0.02	0.04



Figs. 15-23. Scanning electron micrographs of *Gastrocopta* shells. **Figs. 15-19, 21, 25.** *G. gularis,* sp. nov. Figs. 15-17, 19, 25, Paratypes (UF 247776). Figs. 18, 21, specimen from Costa Rica (UF 244467); note the forward projection of the angular lamella in Figs. 16, 17, and 25. **Figs. 20, 22, 23.** *G. hummelincki,* specimens from Margarita Island, Venezuela (UF 246549). Scale = 0.25 mm (19-21, 24-25); 0.5 mm (15-18, 22-23).

Immersidens.

Gastrocopta gularis, G. colombiana, and *G. hummelincki* form a group of closely related species that is ecologically deployed at low-elevation submesic zones in Central America and Caribbean coastal South America. The closest phylogenetic relationship of this species-group appears to be with other South American *Immersidens.*

Etymology. The species name *gularis* is from the Latin, *gula*, and alludes to the obstructed throat of the aperture.

SPECIMENS EXAMINED

Gastrocopta colombiana Pilsbry, 1921
COLOMBIA. Dept. La Guajira: 1 km S of Río Hacho (FMNH 65273, 12 specimens); NE of Río Hacho (FMNH 65396, 3 specimens); 2 km S of Río Hacho (FMNH 65394, 41 specimens).

Gastrocopta dalliana dalliana (Sterki, 1896)
UNITED STATES. Arizona: Cochise Co., Ransey Canyon, Huachuca Mountains (SBMNH 71098, numerous specimens).

Gastrocopta dalliana bilamellata (Sterki and Clapp, 1909)
UNITED STATES. Arizona: Pima Co., Tanque Verde Creek, NE of Tucson (SBMNH 71213, numerous specimens).

Gastrocopta hummelincki Haas, 1960
VENEZUELA. Est. Nueva Esparta: Margarita Island, SE of Valle (ANSP 177413, 14 specimens); Basa del Piache, SE of Valle (FMNH 65270 [holotype], ANSP 178108, 1 specimen); S of La Fuente, Paraguachi (FMNH 65274, 5 specimens); Cerro de Mármoles, E of Guantamare (FMNH 65272, 11 specimens); El Cerrito, E of La Asunción (FMNH 65276, 16 specimens); Morro Grande de Tamarindo (FMNH 65275, 1 specimen); 7 km E of Robledal (11°03.0'N, 64°19.4"W) (UF 246558, *ca.* 100 specimens); Fuentidueño, 2.2 km S of San Juan (11°00.7'N, 63°55.5'W) (UF 246549, *ca.* 100 specimens); Punta Curichicual, 4.4 km SE of Boca de Pozo (10°57.6'N, 64°20.8'W) (UF 246557, 1 specimen); 1 km S of San Francisco (11°00.8'N, 64°17.5'W) (UF 246565, *ca.* 200 specimens). Est. Sucre: Morro de Esmeranda, W of Carúpano (ANSP 177410, 14 specimens).

Gastrocopta iheringi (Suter, 1900)
BRAZIL. Est. Rio Grande do Sul: Bollaxa (ANSP 22940, 1 specimen).

Gastrocopta sp.
TRINIDAD AND TOBAGO. Chacachacare Island (FMNH 65389, 5 specimens).

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LITERATURE CITED

Arias, S. 1955. Los Pupillidae (Pulmonata, Stylommatophora: Orthurethra) colectados en Venezuela septentrional. *Memoria de La Sociedad de Ciencias Naturales La Salle* 15:140-169.

Bequaert, J. C. and W. B. Miller. 1973. *The Mollusks of the Arid Southwest with an Arizona Check List.* University of Arizona Press, Tucson. xvi + 271 pp.

Haas, F. 1960. Caribbean land molluscs: Vertiginidae. *Studies on the Fauna of Curaçao and other Caribbean Islands* 10:1-17, pls. 1-5.

Naranja-Garcia, E. 1991. Present status of the micromollusks of northern Sonora, Mexico. *American Malacological Bulletin* 8:165-171.

Pilsbry, H. A. 1916-1918. Pupillidae (Gastrocoptinae). *Manual of Conchology*, ser. II, 24: i-xii, 1-380, pls. 1-49.

Pilsbry, H. A. 1920-1921. Pupillidae (Vertiginae, Pupillinae). *Manual of Conchology*, ser. II, 26: i-iv, 1-254, pls. 1-24.

Richards, H. G. and P. W. Hummelinck. 1940. Land and freshwater mollusks from Margarita Island, Venezuela. *Notulae Naturae* 62:1-16.

Suter, H. 1900. Observacoes sobre alguns caracóes terréstes do Brazil. *Revista do Museu Paulista* 4:329-337, pl. 3, figs. 8, 8a.

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In situ observations on *Brachioteuthis beanii* Verrill: paired behavior, probably mating (Cephalopoda, Oegopsida)

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Abstract: A behavior that has never been seen in cephalopods was observed three times in a large aggregation of *Brachioteuthis beanii* Verrill, 1881. During a series of submersible dives off Cape Hatteras, North Carolina, eastern U. S., three pairs of *Brachioteuthis* were seen, and one pair was video-taped. In all three pairs one squid grasped the other by the posterior mantle in its arm crown. This paired behavior involved brief periods in which the grasped squid bent its head and body posteriorly and vigorously moved its arms around the head and mantle opening of the grasping squid. Although we were unable to capture any of the coupling pairs to determine their stage of maturity, we believe this unusual activity represents mating behavior.

Key words: Cephalopoda, squid, behavior, mating, *in situ* observations, submersible

The observations reported in this paper are based on a series of dives conducted by the Johnson-Sea-Link I submersible in September 1994 about 95 km off Cape Hatteras, North Carolina, in an area called The Point. The bottom topography is characterized by a series of parallel ridges and canyons that fan out seaward into deep water from the edge of the continental slope. The tops of the ridges where the dives took place were at about 700 m depth, and the walls of the canyons sloped off at angles of 50-70°.

The area of The Point is well known for its extremely high productivity, and the benthic biomass is an order of magnitude greater than in comparable areas (Schaff *et al.*, 1992; Felley and Vecchione, 1995). The ecosystem around The Point is well documented and is the site of ongoing environmental studies, especially as it is a potential location for oil exploration (Diaz *et al.*, 1994). Unpublished reports and video tapes from other submersible operations at The Point confirm that the abundance of cephalopods in the area is very high every year.

Our objectives during the dive series were (1) to test the hypothesis (Trites, 1983; O'Dor and Balch, 1985) that The Point is an area utilized by *Illex* spp. as a spawning ground, and (2) to observe and document occurrence and behavior of all species of cephalopods that were encountered during the cruise. This paper reports the observations on *Brachioteuthis beanii,* particularly on a behavior not previously observed in any species of deep-sea cephalopods. This continues the series of papers from our *in situ* observations on submersible and ROV data (Vecchione and Roper, 1991; Vecchione *et al.*, 1992; Roper and Vecchione, in press).

MATERIALS AND METHODS

The dive sites at The Point were located at 700-1000 m (bottom depth), beyond which the canyon floors sloped seaward into much deeper water. Bottom temperatures were approximately 4.5°C in the canyons.

Of the 15 dives conducted during the cruise, five yielded observations on *Brachioteuthis beanii.* In particular, Johnson-Sea-Link I dive 3745 provided the interesting results described here. This dive took place off Cape Hatteras at The Point on 08 September 1994 (35°13.513' N, 74°57.703' W; 1034-1329 hrs; maximum depth 800 m; surface temperature 28°C; thermocline at 80-120 m spanning 27-17°C; bottom temperature at 800 m, 4.58°C). Specimens of cephalopods were captured by the submersible and by a 2-m Tucker trawl.

Portions of the video sequences upon which this paper is based are presented within the "Cephalopods in Action" web pages through URL = http://nmnhwww.si.edu/cephs/.

RESULTS

In all, 13 species (12 squids and 1 octopod) were observed from the submersible or captured by midwater trawl; large numbers of *Illex* were observed. Major new observations were filmed and recorded for *Mastigoteuthis magna* Joubin, 1913 (Roper and Vecchione, in press) and *Brachioteuthis beanii. B. beanii* was observed on five of the 15 dives at depths of 500 m, 700 m, two at 800 m, and 860 m. One specimen was captured in a midwater trawl, which enabled us to verify the specific identification. Individuals and aggregations occurred from about 5-60 m above the bottom. Most animals were seen in aggregations or schools; as many as 40-60 individuals were in sight from the submersible simultaneously, while others were more scattered. A single individual was seen on only one dive, just a few meters above the bottom. Several *B. beanii* were observed attacking prey consisting of myctophid and sternoptychid fishes.

During JSL-I dive 3745, numerous individuals of *Brachioteuthis beanii* were first encountered at about 700 m in the box canyon with 70° sloping walls. The squids have a golden-colored body; arms and head are pink grading to whitish; the eyes each have a large, elongate anteroventral photophore that reflects the submersible lights brightly.

At about 60 m above the bottom, the submersible encountered loose aggregations of several dozen *Brachioteuthis* that continued to occur down through the rest of the water column. During the final descent to the bottom, two pairs of squid were observed coupled in tandem, as described below. The submersible stopped a few meters above the bottom directly in front of a third pair of already-coupled *B. beanii.* The pair was video-taped for 10 min, during which they remained coupled or in contact.

Because we were not present on that dive, no attempt was made to capture the two squid, each about 20 cm total length, which were still coupled when the submersible started its transect along the bottom. During this and other dives when *Brachioteuthis* were encountered, neither the aggregated individuals nor the three coupled pairs seemed to react to the submersible.

The following description is a summary of the 10-min video tape of the paired *Brachioteuthis beanii* begun after the squid were already coupled. The two were in a tandem position, head-to-tail, with the posterior squid firmly grasping the anterior squid with its arms along the fins and posterior mantle (Fig. 1a). Both squid remained quite still with no apparent attempts by the anterior partner to escape. Infrequently, the posterior squid angled its mantle dorsally, nearly 90° to the axis of the head and arms (Fig. 1b); this right-angle position was held for several seconds. At one point the two squid separated in a flurry of activity with vigorous arm waving and head movement (Fig. 2a). Then they oriented head-to-head, motionless, with their mantles parallel and adjacent for nearly a minute (Fig. 2b), followed by vigorous arm waving. The posterior individual then eased back into the tail-holding position without a struggle. The next sequence involved activity in which the anterior squid underwent gyrations, then a rotating, twisting motion around the longitudinal axis of the body for about 45 sec while being firmly held by the posterior squid. Next followed about 2 min during which the anterior (grasped) squid bent its head and mantle posteriorly, forming nearly a circle, and manipulated its arms vigorously around the head and mantle opening of the posterior (grasping) squid (Fig. 2c). The final minute of observation showed the pair coupled quiescently in the head-to-tail tandem position (Fig. 1a).

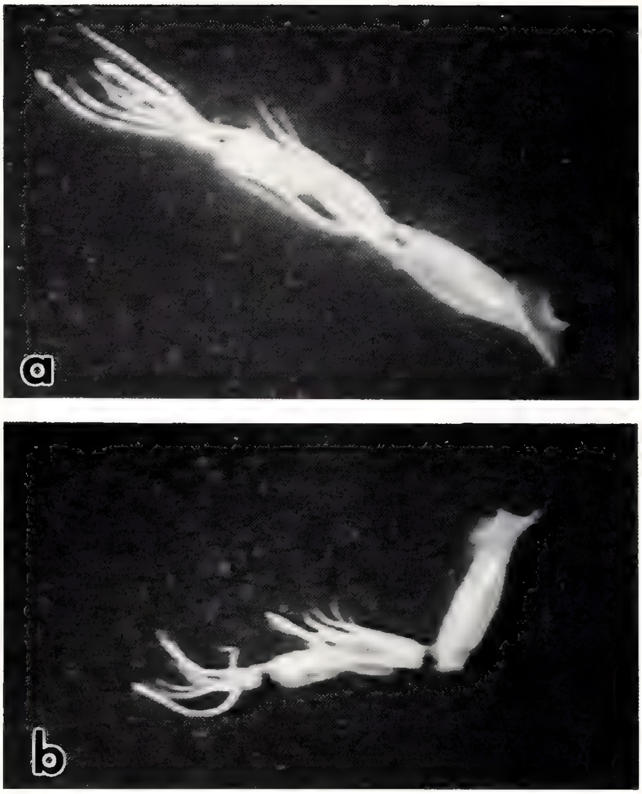


Fig. 1. a. Typical coupling behavior of *Brachioteuthis beanii*, showing posterior squid grasping anterior squid. Three couples were observed in this tandem position. (All figures are photographs made from video tapes). **b.** Coupled pair of *B. beanii* with head and mantle of the posterior squid held briefly in a right-angle position.

Because the animals were not captured and because the resolution of the video tape was insufficient to show details of internal anatomy, we could not determine with certainty the sexes of the observed squid, even though they were of different appearance. The condition of the anterior specimen and visible differences in internal anatomy are worth noting and could provide hints about its sex. Close examination of the specimen in the video revealed that the mantle, fins, and tail are scarred and scratched, undoubtedly a result of being grasped and held by the posterior specimen. Furthermore, the region of the digestive glands and

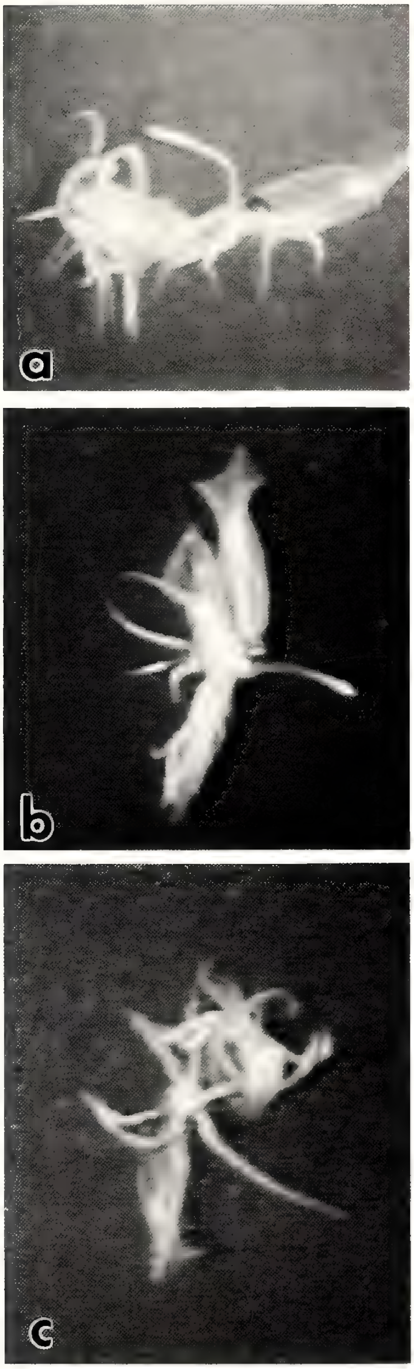


Fig. 2. a. Anterior squid (right) of paired *Brachioteuthis beanii,* waving arms and writhing. **b.** Pair of *B. beanii* lying head-to-head with mantles parallel and adjacent following activity shown in 2a. Anterior squid on left. **c.** Anterior squid (right) of coupled *B. beanii* with head and mantle bent posteriorly and vigorous arm movements.

especially the reproductive glands appears to be different. The major difference is that the anterior specimen has a distinct, broad, whitish, nodular mass dorsal to the digestive gland near the mantle opening (Fig. 3). This could be a mass of spermatophores deposited inside the mantle cavity on the internal mantle wall.

An immature male was captured on a later dive, and the reproductive organs, the spermatophoric apparatus, and testes were developing as asymmetrical structures in the posterior mantle as is shown by the coupled specimen in Fig. 3. There was no trace of a hectocotylus. We examined fully mature males of *Brachioteuthis* in the National Museum of Natural History collection; they had full Needham's sacs and no trace of a hectocotylus, as has been described as a character of the genus. No mature females were available for study and comparison with the video images.

Feeding behavior also was observed in several other *Brachioteuthis beanii* preying on myctophid fish. Further, observations were made on a resting position in which individuals typically were observed. This typical hovering posture has been observed and video taped on several occasions, both during this cruise and previously (unpubl.). The axis of the mantle, head, and arms lies in a horizontal plane; the arms are splayed out laterally in a fan-shaped arc. Very gentle mantle pulsations are seen, or no movement at all, and no forward or rearward locomotion occurs as a result of mantle expulsions. Some motion in the posterior direction occurs from fin flapping, but this is associated mostly with position-holding or slow swimming. During the upstroke the fins overlap dorsally. In some instances, the tentacular clubs are locked together.

Two observations from previous dives in the same area immediately preceding our cruise are relevant to the current discussion. Ophiuroid echinoderms occur in patches of great abundance on the soft sediments of the canyon walls and floor, often so abundant that their arms overlap. One extremely dense patch in this area required 4 min for the submersible to traverse its diameter (Felley and Vecchione, 1995). This phenomenon was observed on our cruise as well. A video sequence showed a cluster of ophiuroids, *Ophiura sarsii* Lütken, 1855, enveloping a *Brachioteuthis beanii* (Fig. 4a). The squid was still alive, as indicated by weak mantle pumping and fin beating, and it lay on its dorsal surface. A second video sequence showed a deep-sea red crab, *Chaceon* cf. *quinquedens* (Smith, 1879), grasping a *B. beanii* in one large cheliped with the posterior end of the squid held in its mouth (Fig. 4b); the maxillipeds were actively working.

DISCUSSION

While the status of the systematics of the Brachioteuthidae is quite confused and the family requires revision, we are confident that the species we observed, video taped, and captured is *Brachioteuthis beanii*, the type

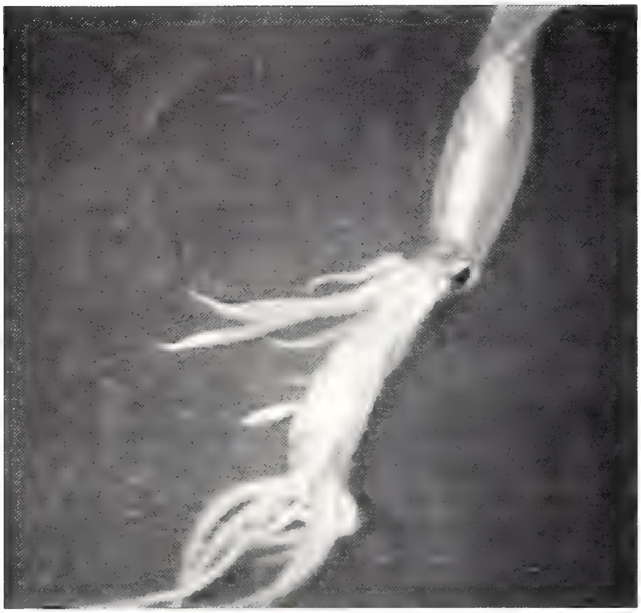


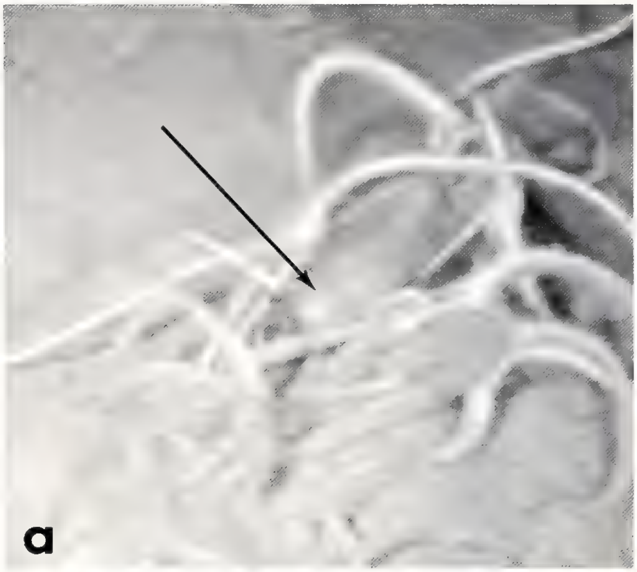
Fig. 3. Coupled pair of *Brachioteuthis beanii* showing different internal structures, having resumed tandem orientation following the activity shown in Figs. 2a-c. Posterior squid (top) with asymetrical reproductive apparatus, probably the male. Anterior squid (bottom) with broad, whitish, nodular mass dorsal to digestive gland in anterior mantle cavity, probably the female.

species of the genus. Our specimen conforms to the description and illustration of this species given by Verrill (1881) for the type specimens caught off Martha's Vineyard. It appears that the species is associated with the Gulf Stream system and that it is the only *Brachioteuthis* species currently known to occur in the northwestern Atlantic Ocean. Even more poorly known is *B. bowmani* Russell, 1909, from the northeastern Atlantic; the relationship between these two taxa is unknown (Nesis, 1987).

Brachioteuthis species are not abundant in collections; collection lots usually contain one or only a few specimens, and very few specimens are sexually mature. At least some species of *Brachioteuthis* develop distinctive dermal structures on the surface of the mantle at maturity (M. Sweeney, pers. comm.), but no such sculpturing was evident on any of the specimens observed during this cruise, nor from videos taken during previous cruises in the area.

In attempting to understand our observations of the previously unobserved coupling behavior in *Brachioteuthis beanii*, we offer two possible explanations:

(1) Cannibalism. The anterior specimen shows evidence of abrasion and external tissue damage on the posterior mantle, fins, and tail. But squids in general, to our knowledge, never have been observed to capture prey in the head-to-tail manner observed here. When squid attack prey, they usually strike in the region of the dorsal head-and-neck junction, to immobilize the prey with a lethal bite. In the three pairs of coupled *Brachioteuthis beanii* observed, the anterior specimen never appeared to struggle or to exhibit strong mantle contractions, as if in an effort to escape. Even when they decoupled and hovered parallel to each other, mantles adjacent and head-to-head, with no grasping action, no efforts to escape were observed. No partially devoured squid were seen. Several *B. beanii* were observed to attack myctophid and sternoptychid fishes which were



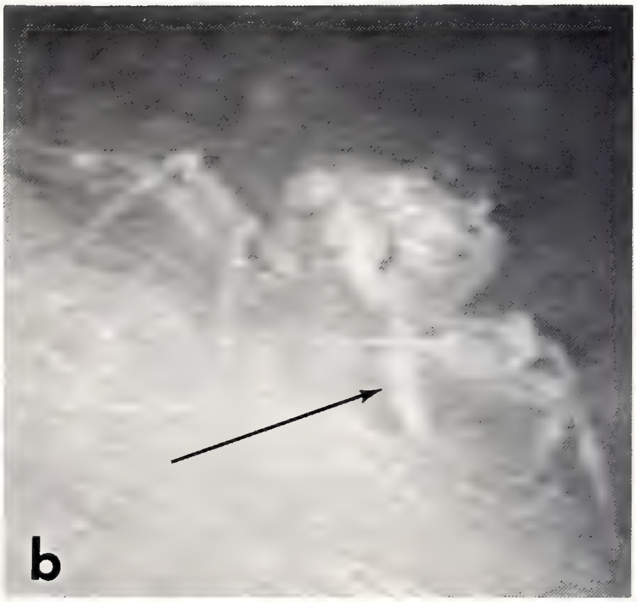


Fig. 4. a. A cluster of brittlestars, *Ophiura sarsii*, enveloping a live but weak *Brachioteuthis beanii* in apparent feeding frenzy. **b.** A deep-sea red crab, *Chaceon* cf. *quinquedens*, feeding on a dead *B. beanii* held in its cheliped.

abundant in the area, providing plenty of food. Cannibalism, therefore, seems unlikely.

(2) Mating behavior. Although the activity we describe here previously has not been observed in any deep-sea squid, we believe the observations can be explained as mating behavior. The coupled specimens were not captured to enable verification of sex and stage of maturity, but our hypothesis is supported by collateral evidence: (a) three pairs of *Brachioteuthis beanii* exhibited identical behavior; (b) the three grasped anterior squid made no attempts to escape; (c) the coupling period was of long duration, with alternating periods of quiescence and strong mutual action of the arms; (d) obvious differences in internal anatomy indicate different sexes; (e) four of the five dives that observed *B. beanii* recorded schools or aggregations of these squids within 60 m of the bottom in a highly productive environment; (f) the tissues of the mantle and fins of the anterior specimen are abraded and damaged, as is well documented for species of squids whose reproductive behavior is known (*e. g.* Hixon, 1983).

The nuchal area is known to be a site for attachment of spermatophores in other oceanic squids, *e. g.* enoploteuthids (Burgess, in press). If the white mass dorsal to the digestive gland observed in our video tape is a deposit of spermatophores, the coupled specimens are different sexes and the anterior specimen would be the female, while the posterior specimen, with its asymetrical reproductive apparatus, would be the male. The female would not have to be fully mature to mate and receive spermatophores; this would be similar to the case in *Illex coindetii* (Verany, 1839) (Mangold-Wirz, 1963). It could be a selective/evolutionary advantage for an early-maturing male to have his spermatophores in place and available for fertilization of the eggs the moment they become ripe. Retention of viable sperm is known in females of other species of cephalopods (Mangold, 1987).

Scavenging by ophiuroids and a *Chaceon* crab on weak or dead *Brachioteuthis beanii* is additional evidence that spawning followed by death occurs in this area. There seems no possibility that ophiuroids or *Chaceon* crabs could capture a healthy mesopelagic or bentho-pelagic squid, even if it were resting on the bottom. Although *Illex* commonly rests on the bottom, we never have observed *Brachioteuthis* to do so. We suggest that the squid were spent and dying individuals that had sunk to the bottom after spawning as is known for other cephalopods (Mangold, 1987). Spent, dying squids that sink to the bottom could provide a significant source of energy to the deep benthic fauna.

We believe that the most reasonable explanation for these observations on *Brachioteuthis beanii* is that they demonstrate, for the first time, mating behavior in deep-sea squids.

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LITERATURE CITED

Burgess, L. A. In press. A survey of seminal receptacles in the Enoploteuthidae. *In: Systematics and Biogeography of Cephalopods,* N. A. Voss *et al.*, eds. *Smithsonian Contributions to Zoology.*

Diaz, R. J., J. A. Blake, and G. Randall Cutter, Jr., eds. 1994. Topical studies in oceanography. Input, accumulation and cycling of materials on the continental slope off Cape Hatteras. *Deep-Sea Research II* 41(4-6):705-982.

Felley, J. D. and M. Vecchione. 1995. Assessing habitat use by nekton on the continental slope using archived videotapes from submersibles. *Fishery Bulletin* 93(2):262-273.

Hixon, R. 1983. *Loligo opalescens. In: Cephalopod Life Cycles. Vol. I, Species Accounts,* P. R. Boyle, ed. pp. 95-114. Academic Press, London.

Mangold, K. 1987. Reproduction. *In: Cephalopod Life Cycles. Vol. II, Comparative Reviews,* P. R. Boyle, ed. pp. 157-200. Academic Press, London.

Mangold-Wirz, K. 1963. Biologie des cephalopodes benthiques et nectoniques del la Mer Catalane. *Vie et Milieu (suppl.)* 13:1-285.

Nesis, K. N. 1987. *Cephalopods of the World. Squids, Cuttlefishes, Octopuses, and Allies.* T. F. H. Publications, Neptune City, New Jersey, 351 pp.

O'Dor, R. K. and N. Balch. 1985. Properties of *Illex illecebrosus* egg masses potentially influencing larval oceanographic distribution. *Northwest Atlantic Fisheries Organization, Scientific Council Studies* 9:69-76.

Roper, C. F. E. and M. Vecchione. In press. *In situ* observations test hypotheses of functional morphology in *Mastigoteuthis* (Cephalopoda; Oegopsida). *Vie et Milieu.*

Schaff, T., L. Levin, N. Blair, D. DeMaster, R. Pope, and S. Boehme. 1992. Spatial heterogeneity of benthos on the Carolina continental slope: large (100 km)-scale variation. *Marine Ecology Progress Series* 88:143-160.

Trites, R. W. 1983. Physical oceanographic features and processes relevant to *Illex illecebrosus* spawning in the western North Atlantic and subsequent larval distribution. *North Atlantic Fisheries Organization, Scientific Council Studies* 6:39-55.

Vecchione, M. and C. F. E. Roper. 1991. Cephalopods observed from submersibles in the western North Atlantic. *Bulletin of Marine Science* 49(1-2):433-445.

Vecchione, M., B. H. Robison, and C. F. E. Roper. 1992. A tale of two species: tail morphology in paralarval *Chiroteuthis* (Cephalopoda: Chiroteuthidae). *Proceedings of the Biological Society of Washington* 105(4):683-692.

Verrill, A. E. 1880-1881. The cephalopods of the north-eastern coast of America. Part II. The smaller cephalopods, including the "squids" and the octopi, with other allied forms. *Transactions of the Connecticut Academy of Sciences* 5(6):259-446.

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Male reproductive anatomy of *Vitreledonella* (Cephalopoda: Octopoda)

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Abstract: The hectocotylus and the internal reproductive anatomy of a nearly mature male specimen of *Vitreledonella richardi* Joubin, 1918, are described. Distally, the third left arm carries a spermatophore groove, a ventrally open spherical structure and an elongate arm tip. Notable among the internal reproductive organs are the large ampulla and the elongate accessory gland. Incomplete reports of the few specimens have limited our knowledge of *Vitreledonella* and other groups of mid-water octopods. Critical to advancing our knowledge is sharing data derived from the few available specimens.

Key words: *Vitreledonella*, hectocotylus, male reproductive anatomy

A lanceolate digestive gland, reduced digestive organs that are rotated cephalad, and a octopus-like radula are characters that Thore (1949) used to delineate the genus *Vitreledonella* Joubin. In the description of *V. richardi* Joubin, 1918, the type species and the only one currently recognized as valid, Joubin (1918) detailed virtually every aspect of the single female specimen known to him. Despite the traditional emphasis of octopod taxonomy on male reproductive characters, male anatomy of *Vitreledonella* remains virtually unknown. To my knowledge, only three hectocotyli of *Vitreledonella* have been reported in any detail in the literature, two from specimens collected off the western coast of Africa (Robson, 1930: fig. 9; Adam, 1983), and one from east of Australia (Thore, 1949: fig. 53). The modified (suckerless) portion of each of these is reported to be less than 3 mm long and to lack the modifications reported in the specimen described here.

This report describes the nearly mature hectocotylus and internal reproductive organs of a single male specimen of *Vitreledonella*. The specimen is assumed to represent *V. richardi* which is reported to range from 48° N to 33°S and to depths of over 1750 m in the Pacific, Atlantic, and Indian Oceans (Thore, 1949).

MATERIALS & METHODS

Male specimens examined are listed in Table 1. Specimens were identified to genus by the apomorphic characters of the lanceolate digestive organ and the very strong reduction of the digestive organs and the inner gill lamellae. The plesiomorphic open mantle aperture near the funnel distinguishes *Vitreledonella* from *Amphitretus* Hoyle (both share the cephalad rotation of the digestive system). Measurements and counts that have been standardized for benthic octopuses (Robson, 1929; Toll, 1988) were made on all specimens. On specimens in which the arms were intact, arm length, sucker diameter, and sucker counts are reported for the second left arm. For specimens in which arm suckers were missing, but the arms were otherwise intact, sucker ganglia were counted to estimate sucker number.

One male specimen (UMML 31.1566), represented by physically separate mantle and arm crown, had been collected from the stomach of an *Alepisaurus* (lancet fish). The hectocotylus of this specimen was comparatively developed. The reproductive organs were illustrated *in situ*, then removed from the mantle cavity of this specimen and the superficial membrane, the genital sac, that bound them. The organs were dissected apart. Their relative sizes and arrangements were illustrated using a camera lucida attached to a Wild dissecting microscope.

Terminology applied to male reproductive organs has been inconsistently applied to cephalopods. The term hectocotylus, as used here, refers to one of the third arms that is modified, presumably for spermatophore transfer. The homologies between the structures on the hectocotylus described here and the ligula and calamus of the Octopodidae remain unclear, precluding the use of these terms. The terminology of Peterson (1959) is used for the internal reproductive organs.

RESULTS

In males of *Vitreledonella*, primary sexual dimorphism consists of modifications to the third left arm to form

Table 1. Male specimens examined with catalogue number (FMNH, Field Museum of Natural History; UMML, University of Miami Marine Laboratories; BMNH, The Natural History Museum, London), locality, depth, collection date, and measurements (in mm) and number of suckers and gill lamellae. (– = data unavailable due to specimen damage; * = mantle and head detached from arm crown; h = holotype of *V. translucida*).

	FMNH	UMML	UMML	BMNH
Catalog No.	78335	31.1467	31.1566	1931.1.21.3^h
Locality	32° 11.7'N 64° 44.5'W	12° 3'-6.2'N 28° 51'-42.6'W	33° 16.9'S 94° 25.0'W	9° 38'00"S 12° 42'30"E
Depth (m)	730-820	170	ex. *Alepisaurus*	200-230
Date	July 1948	Aug. 1973	Jan. 1963	July 1927
Mantle Length	27.4	20.5	39.1*	35.8
Mantle Width	24	10.8	22.0	25.0
Head Width	17.8	12.9	20.5	16.9
Arm II Length	ca. 28	–	92	34.0
Sucker Diameter	–	–	3.5	1.15
Sucker Count	–	–	30	32
Gill Count	7	6	7	7

the hectocotylus. Compared to the third right arm, the hectocotylus is shorter, carries four or five fewer suckers (Table 2) and, distally, has smaller suckers which are more crowded.

In only one male specimen (UMML 31.1566) was the hectocotylus well-developed, *i. e.* with a modified tip longer than 6 mm and structures differentiated between the tip and the suckered portion of the arm. This description pertains to this specimen, despite its collection from the stomach of a predatory fish.

A thin, smooth groove follows the ventral margin of the arm along the most distal suckers (Fig. 1A); whether the left web sector D is modified cannot be ascertained as it, and the fourth arm pair, are missing. The ventral margin of this groove for a width of 1 mm shows clearly defined musculature; the inner portion of the groove, between the margin and the arm, is thin. These characters suggest that this is the spermatophore groove.

Distal to the terminal sucker is a large, rigid, nearly spherical structure, 1.5 mm in maximum diameter; its ventral opening is continuous with the spermatophore groove (Fig. 1B). On the aboral surface, a thin, filmy layer or coating of very delicate skin encircles the structure (Fig. 1C). Orally, the sphere's elongate opening runs parallel to the length of the arm and terminates in a pleated semi-circle. Wholly within the sphere is a projection that extends the diameter of the sphere. Distal to the spherical structure, the arm tip tapers gradually to its tip (Fig. 1A). The arm tip extends around the aboral surface of the spherical structure, before straightening to parallel the axis of the arm (Fig. 1C).

The gonad and associated male organs are located on the dorsal surface of the viscera within the mantle cavity. The testis lies immediately dorsal to the spiral caecum, and just behind the stomach; it and associated organs extend into the mantle cavity, away from the head and mantle aperture, along the digestive gland (Fig. 2A). The single, medial testis is partially covered by the very large accessory gland. The penis and its diverticulum are nearly opposite the systemic heart, positioned very far from the mantle aperture (Fig. 2A).

The single, cream-colored, firm-textured testis apparently empties sperm into the coelom around the testis that is drained by a well-developed, ribbed ampulla (Fig. 2B). The vas deferens appears to be short, although it was not dissected out, and is continuous with the mucilaginous gland, identified by the thickening distal to the ampulla's insertion. The comparatively straight spermatophoric gland inserts into the exceptionally long accessory gland (Fig. 2B). The accessory gland, composed of orange glandular

Table 2. Mensural (in mm) and meristic characters of the modified third left arm from specimens reported here and from literature accounts. (– = data unreported).

	Mantle Length	Total Length	Hecto. Length	Arm Tip Length	No. of suckers on Hectocotylus
FMNH 78335	27.4	56	21	1.12	28
BMNH 1931.1.21.3	35.8	72.3	35.8	1.15	27
Adam (1983)	60	137	50	–	23
Thore (1949)	32	100	59	2.3	–
UMML 31.1566	39.1	131	59	6.1	26

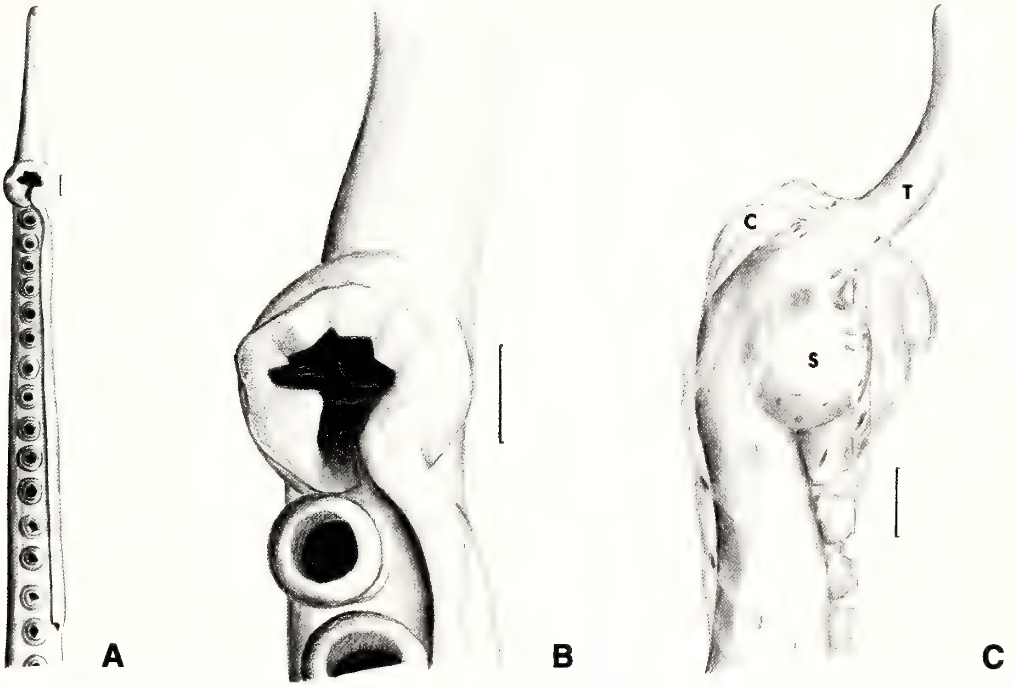

Fig. 1. Three views of the hectocotylus of *Vitreledonella richardi* (UMML 31.1566). A. Oral view of the terminal 16 suckers, the spherical structure, and the arm tip. B. Enlarged view of the spherical structure. C. Dorso-lateral view of the spherical structure (S), its coating (C), and the arm tip (T). Scale bars = 0.6 mm.

tissue throughout, is the longest and widest section of the reproductive organs. This blind-ended gland follows a straight course for 15 mm as it narrows and folds back on itself for 9 mm (Fig. 2B). At its tip, the accessory gland empties into one side of Needham's sac, a straight, thin-walled tube located close to the entrance of the spermatophore duct. Externally visible bands on the apparent spermatophore storage organ reflect internal trabeculae that appear to divide the sac into two parts, as Peterson (1959) described. The sac is continuous with the penis and its diverticulum.

The penis and diverticulum are retort-shaped, as reported in the description of *Vitreledonella translucida* Robson, 1930. Their lengths are subequal (penis length 3.1 mm; diverticulum length 2.6 mm). Although the reproductive organs are enlarged, no spermatophores are present.

DISCUSSION

Previous reports of the short hectocotylus of males of *Vitreledonella* (Table 2) could have led workers to assume that the typical hectocotylus of the genus is similar to that of octopodids as early in its development, the arm tip superficially resembles the ligula and calamus of the octopodids (Robson, 1930: fig. 9; Thore, 1949: fig. 53). The elongate arm tip and the conspicuous spherical structure of the hectocotylus illustrated here (Fig. 1), however, is clearly distinct from any known in the Octopodidae. Robson (1930) noted a similarity between the hectocotyli of *V. translucida* and that of *Amphitretus*, as illustrated by Sasaki (1917: fig. 2), despite the small size of the hectocotylus he examined (Table 2). My examination of various hectocotyli of *Amphitretus* spp. suggests that the principal difference lies in *Vitreledonella* having a smaller hectocotylus, and a spherical structure with a projection located internally. In specimens of *Amphitretus*, the structure appears to be compressed and the projection extends distally beyond the structure. The two families had been assigned to distinct groups, because radular differences were felt to be more important than their shared possession of the cephalad rotation of the viscera (Thore, 1949).

Despite the similarity of the internal male reproductive organs of *Vitreledonella* to those that have been documented in other incirrate octopods (Marchand, 1907; Peterson, 1959), two organs are unusual. First, the ampulla, which moves sperm from the testis to the accessory organs, is similar in size and angle of insertion to that in bolitaenids (Chun, 1910). Second, the accessory gland is extremely elongate. Until we have a better knowledge of the function of this latter organ, or at least a formed spermatophore to examine, the significance of this elongation will remain unknown.

Except for gravid females, adults of *Vitreledonella* are rare in collections, a problem that continues to limit our

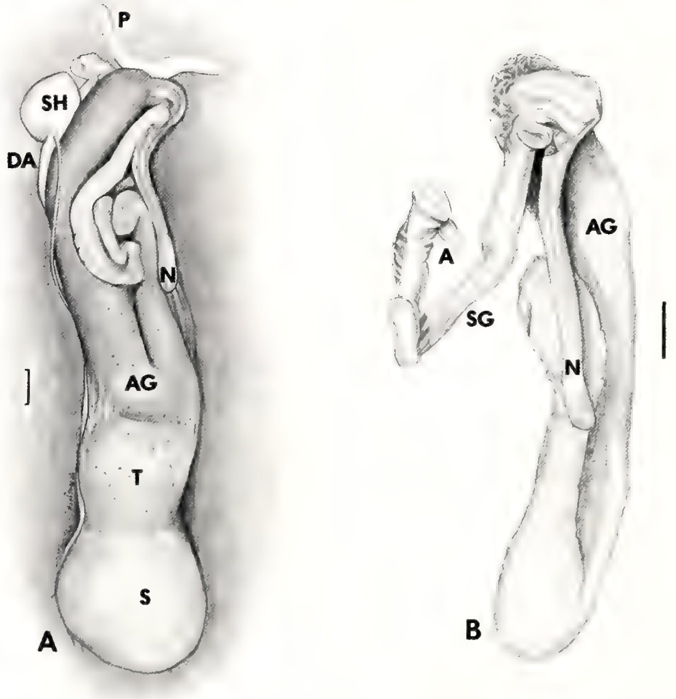

Fig. 2. *Vitreledonella richardi* (UMML 31.1566) A. *In situ* view of the dorsal surface of the viscera in the mantle cavity showing the male reproductive organs. The brain (not illustrated) is located just off the stomach at the lower end of the illustration; the gills lie lateral to the systemic heart and the proximal Needham's sac. B. Ventral lateral view of male reproductive organs exclusive of the genital sac and testis; cephalad is at the bottom of the illustration. Because the spermatophore duct, accessory gland, and Needham's sac all meet in a very small area, that area was not illustrated. (A = ampulla; AG = accessory gland; DA = dorsal aorta; N = Needham's sac; P = penis; S = stomach; SG = spermatophoric gland; SH = systemic heart; T = testis). Scale bars = 0.6 mm.

knowledge of the genus. This description of the most mature hectocotylus known to date in the genus and the compilation of comparative data (Table 2) aim to improve our species-level knowledge of the group. Species boundaries in the genus have been confused because the first five specimens discovered were described as four distinct species which were synonymized without examination of the types (Thore, 1949). Differences among the five males in Table 2 in the number of suckers on the hectocotylus, a character that can help to separate species among the octopodids (Toll, 1988), could be argued to support the hypothesis that more than one species exists in the genus.

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LITERATURE CITED

Adam, W. 1983. Cephalopoda from West and South Africa. *Atlantide Report* 13:151-180.

Chun, C. 1910. The Cephalopoda. Part II. Myopsida, Octopoda. *Scientific Results of the German Deepsea Expedition on Board the Steamship "Valdivia."* Translated by Israel Program for Scientific Translations, 1975, 436 pp.

Joubin, L. 1918. Études préliminaires sur les céphalopodes recueillis au cours des croisières de S. A. S. le Prince de Monaco. 6th Note: *Vitreledonella richardi* Joubin. *Bulletin de l'Institut Océanographique* 340:1-40.

Marchand, W. 1907. Studien über Cephalopoden. I. Der Männliche Leitungsapparat de Dibranchiaten. *Zeitschrift für Wissenschaftliche Zoologie* 86:311-415.

Peterson, R. P. 1959. The anatomy and histology of the reproductive systems of *Octopus bimaculoides. Journal of Morphology* 104:61-82.

Robson, G. C. 1929. *Monograph of the Recent Cephalopoda. I. The Octopodinae.* British Museum of Natural History, London. 236 pp.

Robson, G. C. 1930. Cephalopoda, I. Octopoda. *Discovery Reports* 2:371-402.

Sasaki, M. 1917. Notes on the Cephalopoda, I: On the males of *Amphitretus pelagicus* Hoyle. *Annotationes Zoologicae Japonenses* 9:361-364.

Thore, S. 1949. Investigations on the "Dana" Octopoda, Part I. Bolitaenidae, Amphitretidae, Vitreledonellidae and Alloposidae. *Dana Report* 33:1-85.

Toll, R. B. 1988. The use of arm sucker number in octopodid systematics (Cephalopoda: Octopoda). *American Malacological Bulletin* 6:207-211.

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Laboratory observations on *Todarodes pacificus* (Cephalopoda: Ommastrephidae) egg masses

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Abstract: Two egg masses of the ommastrephid squid *Todarodes pacificus* (Steenstrup, 1880) are described. Immature squid were collected from inshore waters of southern Hokkaido, Japan, and maintained in a raceway tank where they matured, mated, and spawned. Both gelatinous masses were spherical and nearly neutrally buoyant. The larger mass measured 80 cm in diameter and contained approximately 200,000 eggs. The egg-mass surface layer effectively prevented crustaceans, protozoans, and bacteria from infesting the masses. Paralarvae hatched after 4-6 days at 18-19°C and actively swam at once, with many individuals swimming at the surface. Both masses disintegrated soon after hatching. Paralarvae died approximately 6-7 days after hatching, presumably due to starvation.

Key words: reproduction, eggs, squid, *Todarodes*, Cephalopoda

The ommastrephid squid *Todarodes pacificus* (Steenstrup, 1880) is a commercially important resource in Japan, occurring throughout Japanese coastal waters (Okutani, 1983; Murata, 1989, 1990). Many studies have focused on the fishery biology of *T. pacificus* (Murata *et al.*, 1971, 1973; Tashiro *et al.*, 1972; Araya, 1976; Murata, 1978); little, however, is known about reproduction.

Ommastrephid squids, where known, generally produce large numbers of small eggs, encapsulated in gelatinous masses (O'Dor *et al.*, 1982a). Few records exist of naturally spawned eggs or egg masses from oceanic cephalopods (Sabirov *et al.*, 1987; Lapitkhovsky and Murzov, 1990), and there have been no observations of *Todarodes pacificus* egg masses in the natural habitat. [Egg masses found near the Kuril Islands and south of Japan, and attributed to *T. pacificus* by Akimushkin (1963) were presumably misidentified (Kir Nesis, pers. comm.).] All information of this critical period of the life cycle comes from laboratory observations of spawning by captive *T. pacificus*.

Spawning in captivity by *Todarodes pacificus* was first observed by Hamabe (1961a), who suggested that egg masses of *T. pacificus* are normally demersal, and either attached to the sea bottom or deposited in crevices. Hamabe kept spawning females in small barrels anchored on the sea bottom at depths of 5-20 m and obtained 15 egg masses, with each mass containing 300-4,000 eggs. Hamabe (1963) further described broken and incomplete masses spawned in small (volume < 0.13 m^3) laboratory tanks.

Observation of the spawning of a captive *Todarodes pacificus* female by one of us (YS) and our colleague Y. Ikeda in 1991 revealed that *T. pacificus* can produce gelatinous egg masses that resemble those of captive *Illex illecebrosus* (LeSueur, 1821) (O'Dor and Balch, 1985). This paper describes the second such spawning by captive *T. pacificus* and is the first published description of a complete egg mass spawned by this species under laboratory conditions.

MATERIALS AND METHODS

On 1 September 1994, 20 immature squid (mean dorsal mantle length (DML) *ca.* 20 cm) were collected with automatic jigging machines and by hand jigging from the inshore waters of Tsugaru Strait, southern Hokkaido, Japan, and transferred to the Usujiri Fisheries Laboratory, Hokkaido University. The squid were maintained in a filtered, recirculating raceway tank (5.5 m in length, 2.5 m in width, 1.2 m in depth, and 13,000 l in capacity). The maintenance procedure followed that described by Sakurai *et al.* (1993). The squid were maintained for 25 days at a mean temperature of 17.3°C (range 15.8-18.5°C) while they matured and mated, and were fed a daily diet of frozen Pacific saury [*Cololabis saira (*Brevoort, 1850)], and Japanese anchovy [*Engraulis japonicus (*Temminck and Schlegel, 1846)]. The maturity and condition of the squid were monitored daily.

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On 16 September, when mature eggs were first observed in the oviducts, all but four ripe females were removed from the tank. Water circulation was weakened and aeration was turned off to prevent possible damage to egg masses. Two females spawned incomplete egg masses and died on 24 September, leaving two mature females. The DML of the two remaining females measured 26.5 cm and 27.0 cm. Two egg masses were discovered on the morning of 25 September. The masses were maintained a mean temperature of 18.7°C (range 18.3-19.2°C). To facilitate the moving, viewing, and photographing of the masses, the smaller mass was held in a plankton-net container with a plastic window, and the larger mass was held in a gill net (mesh size 49 mm in stretch length) suspended from the surface. Both masses were photographed with a 35-mm camera and videotaped with a Sony CCD-V5000 video camera and an Olympus endoscope. Distances between eggs and fertilization rates within each mass were determined from the videotaped recordings. The mean inter-egg distance was used to estimate the total number of eggs in each mass.

Daily observations were made of the paralarvae. Feeding was not attempted. Individuals were removed daily for future scanning electron microscopic and statolith analysis. Paralarvae were maintained at a mean temperature of 18.8°C (range 18.4-19.2°C).

RESULTS

Spawning females

About two days before spawning, the two females stopped feeding and often rested on the tank bottom. While resting, the females' chromatophores flashed rapidly over the entire body surface in a characteristic, incandescent pattern that is indicative of imminent spawning (YS, pers. obs.). This behavior continued through spawning. Both females died within 12 h after spawning. Postmortem examination of the oviducts revealed neither female spawned all of her eggs. The 27-cm and 26.5-cm DML females had approximately 110,000 eggs and 93,000 eggs, respectively, remaining in the oviducts after spawning. The anterior ends of the nidamental glands from both females were attenuated and slightly translucent.

Egg masses

Both spherical egg masses were nearly neutrally buoyant and found floating near the surface. Both were attached to fragments of previously spawned incomplete masses floating at the surface. The two layers of the egg mass described by Hamabe (1961a, 1963) were clearly visible. Externally, the masses were covered with a jellylike secretion, presumably from the nidamental gland, and the interior of the masses, which contained the eggs, consisted of a jelly presumably secreted by the oviducal gland.

The larger egg mass measured 80 cm in diameter and contained approximately 200,000 eggs (Fig. 1A). More than 90% of the eggs within the mass were fertilized, with localized areas of unfertilized eggs evident within the mass. Infertile eggs were translucent. One side of the mass was damaged, and the jelly forming the surface layer was missing in this area; eggs from the inner core appeared to exude from this area. The smaller egg mass measured 40 cm in diameter and contained approximately 21,000 eggs. Percent fertilization of eggs within this mass was approximately 95%. All developing embryos within both stationary masses underwent development in a vertical position, with the tail pointed upwards. Eggs were positioned 0.4-2.0 cm apart throughout the inner mass. The chorion surrounding each egg expanded to diameters of 1.9-2.3 mm before hatching.

Examination of the egg-mass surface layer revealed that the outer nidamental-gland jelly was effective in preventing crustaceans, protozoans, and bacteria present in the tank from infesting the egg masses (Fig. 1B). Sections of the larger mass where the outer jelly was damaged or missing were quickly infested by bacteria and protozoans. Fragments of nidamental-gland jelly with attached eggs, presumably from the large mass or a previous failed spawning, were also seen floating at the surface. The buoyancy of these fragments was due to embedded air bubbles from the tank's water circulation system. Most of these eggs were quickly infected and died.

The small and large egg masses completely disintegrated six and seven days after spawning, respectively (Fig. 2). After disintegration, pieces of the surface-layer jelly were found on the bottom of the tank. Several pieces with a few unhatched eggs attached and with embedded air bubbles were found floating at the surface.

Paralarvae

Hatching occurred 4-6 days after spawning at *ca.* 19°C. This development rate was similar to that described by Hamabe (1961b) for *Todarodes pacificus* (4-5 days at 15-20°C). The DML of hatchling paralarvae measured 1.1 mm. At hatching, paralarvae appeared to swim vertically out of the egg masses. Once free, they swam freely in the tank, with many at the surface. No paralarvae appeared to remain within either mass after hatching.

Two general swimming patterns were seen. Many paralarvae followed a general pattern of slow ascent from midwater in the tank until they reached the surface, where they swam in circular or random patterns at the surface. These paralarvae spent a long period swimming at the surface, after which they would cease swimming and sink from the surface. Another pattern seen in several paralar-

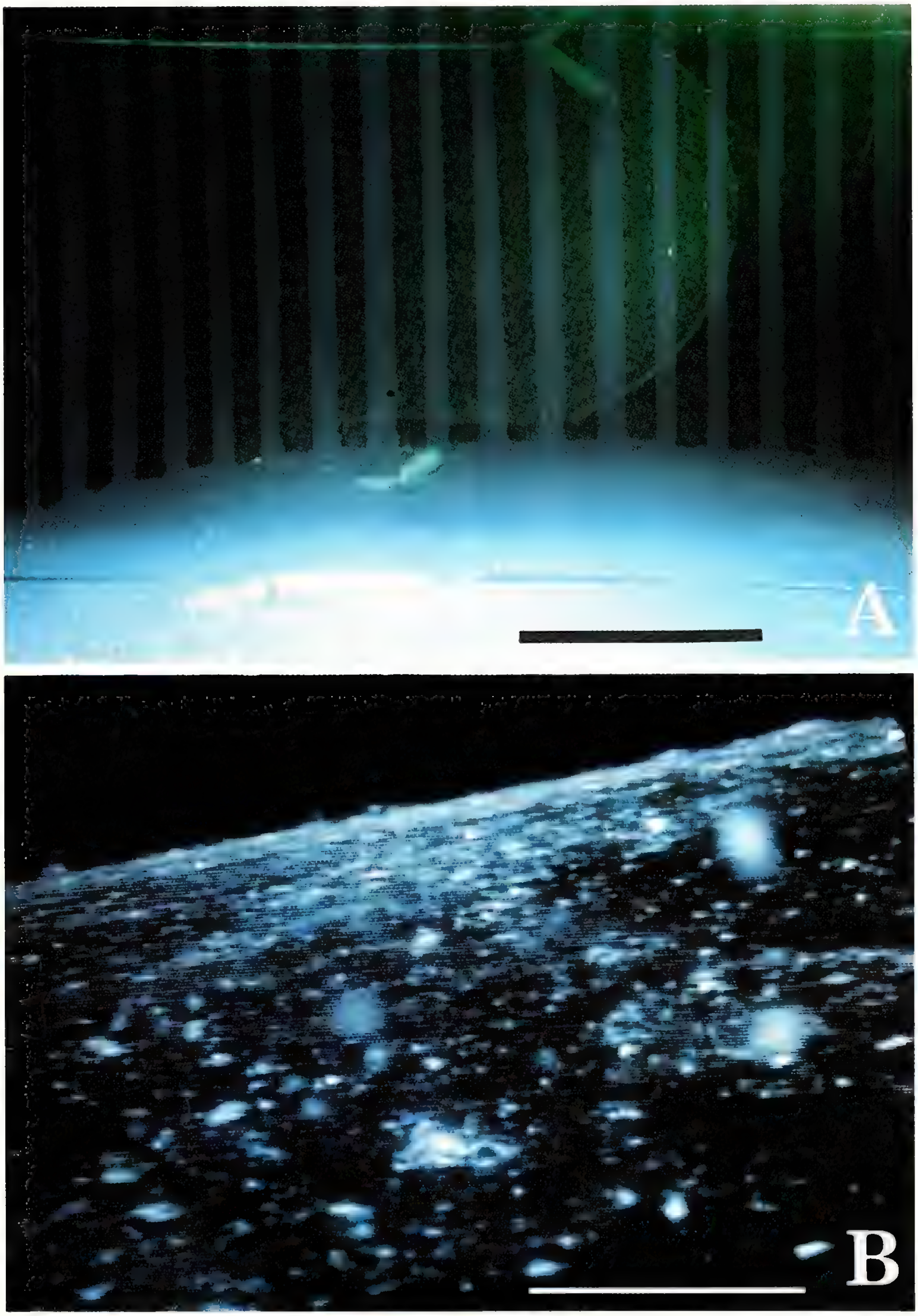

Fig. 1. Egg masses of *Todarodes pacificus*. **A.** The floating 80-cm-diameter spherical egg mass spawned in a laboratory tank. Positive buoyancy was due to attached fragments from previously spawned masses floating at the surface. Note the two dead spawned females on the tank bottom. Scale bar = 50 cm. **B.** Top surface of the 40-cm-diameter egg mass. Crustaceans, protozoans and bacteria visible on the outer nidamental-gland jelly layer of the egg mass could not infest the egg mass. Scale bar = 5 mm.

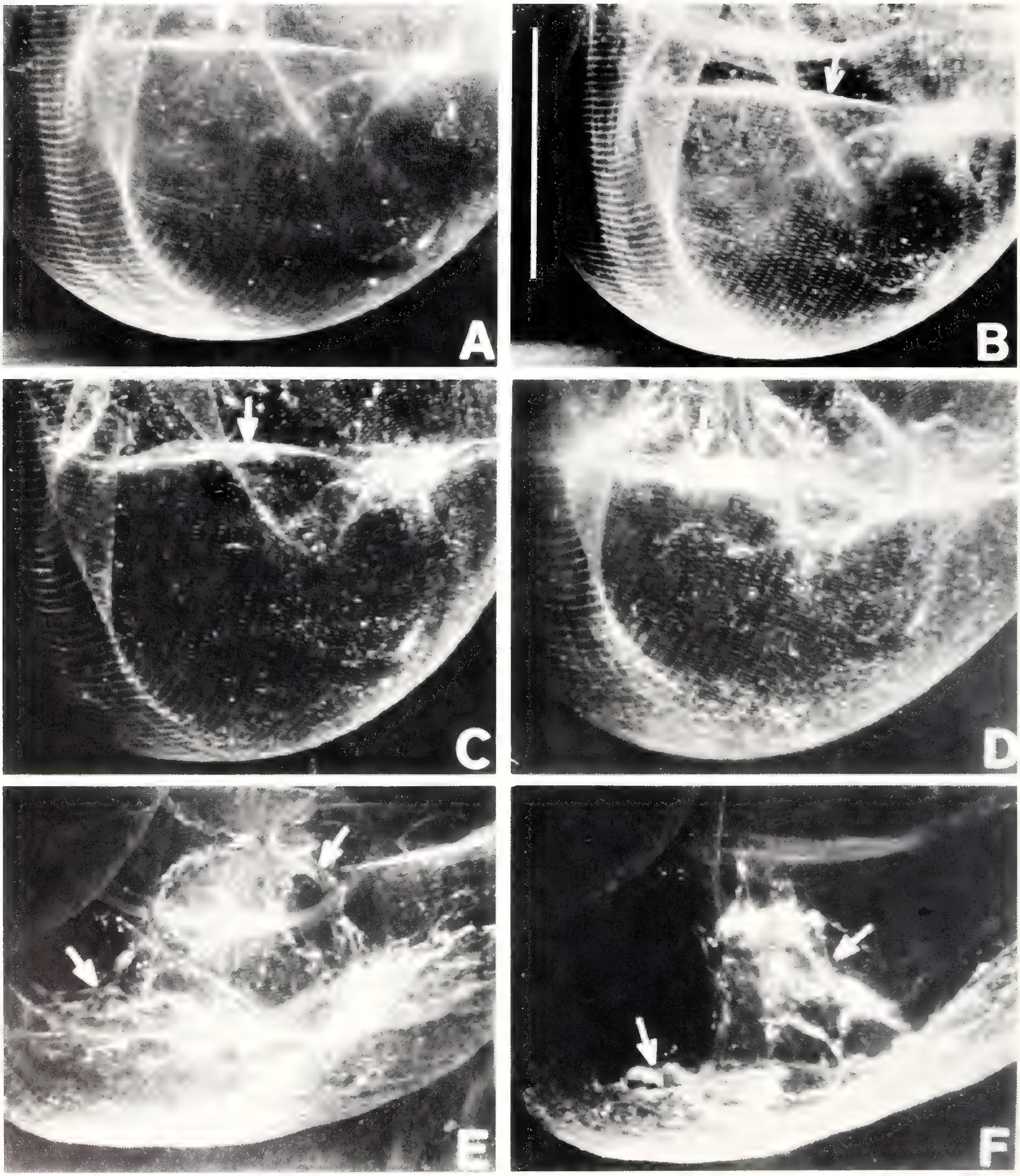

Fig. 2. Time series showing the 80-cm-diameter egg mass held in a gill net suspended from the surface. Scale bar = 50 cm (A-F). **A.** 2 days after spawning. **B.** 3 days after spawning. **C.** 4 days after spawning. **D.** 5 days after spawning. **E.** 6 days after spawning. **F.** 7 days after spawning. Arrows indicate outline of egg mass.

vae was a slow ascent to the surface, cessation of swimming once the surface was reached, followed by immediate sinking. Feeding by the paralarvae was not observed. Paralarvae swam with rapid mantle contractions of *ca.* 125 beats per minute (normal adult rate = 75 beats per minute). While swimming at the surface, many paralarvae had unidentified particles stuck to their mantle surface. Two paralarvae were also seen swimming with mucus strands approximately 2 cm in length trailing from the mantle surface.

No pattern of phototaxis was seen; paralarvae were found at the surface in uniform numbers both day and night. When we shined lights directly on the paralarvae at the surface while photographing, however, the paralarvae avoided the strong light by sinking in a somersault motion. Paralarvae at the surface displayed positive rheotaxis when a weak flow was generated from a bubbling stone.

Paralarvae died approximately 6-7 days after hatching, presumably due to starvation.

DISCUSSION

Spawning females

The observed resting behavior on the bottom by mature females before spawning appears to support Hamabe's (1961a, 1963) suggestion that *Todarodes pacificus* is a demersal spawner. The female observed by Hamabe spawned while resting on the bottom of a barrel. Such resting behavior has been reported in other neritic ommastrephids (Bradbury and Aldrich, 1969; Boletzky *et al.,* 1973; O'Dor and Balch, 1985; Vecchione and Roper, 1991; Young, 1995). However, while spawning could occur near the bottom in shallow inshore waters, *T. pacificus* likely spawns at the same depths when over deep waters, which would ensure that the nearly neutrally buoyant egg masses are maintained in the warm surface layer, where temperatures are adequate (above 12^{O}C; Sakurai *et al.,* 1996) for normal embryonic development. The delicate nature of the masses indicates that they are not attached to the sea bottom, as Hamabe proposed.

Our findings suggest that captive post-spawning females die with many eggs still remaining within their oviducts. Such incomplete spawning is common in captive, spawned females (Ikeda *et al.*, 1993). In November 1994, a pre-spawning female (DML = 26.8 cm) had *ca.* 304,000 eggs within her oviducts (JB, pers. obs.). This count is comparable to the figures of Soeda (1956), who estimated the fecundity of *Todarodes pacificus* to be between 320,000 and 470,000 eggs. Future fecundity estimates of *T. pacificus* must consider that females do not necessarily spawn all eggs before dying.

Egg masses

Our observations demonstrate that *Todarodes pacificus* can produce nearly spherical egg masses up to 80 cm in diameter, with *ca.* 200,000 eggs. Hamabe (1961a) obtained 15 small egg masses, with each mass containing 300-4,000 eggs, however, the small size of the barrels used during his experiment (barrel length = 50 cm; barrel inner diameter = 33 cm) presumably prevented the formation of a complete mass.

Egg masses formed by *Todarodes pacificus* resemble those formed by the ommastrephid *Illex illecebrosus* (Durward *et al.,* 1980; O'Dor and Balch, 1985). Percent fertilization within the *T. pacificus* masses, however, was significantly higher than the maximum of 40% reported for *I. illecebrosus* egg masses (O'Dor *et al.,* 1980). The main difference during spawning between these species is the manner in which fertilization occurs. During egg-mass formation by *T. pacificus*, sperm from the seminal receptacles, located on the buccal membrane, must pass through the nidamental gland jelly and mix with the oviducal jelly and eggs in the inner layer of the egg mass. An uneven flow of sperm from the seminal receptacles to the egg mass could account for the localized variability in fertilization rates within the large egg mass. In contrast, *I. illecebrosus* has no seminal receptacles, and fertilization occurs when females form a mixture of concentrated jelly (nidamental and oviducal), eggs and broken spermatophores within the mantle cavity (Durward *et al.,* 1980).

A notable difference in embryonic development rates was found between *Todarodes pacificus* eggs that developed within an egg mass and those reared by artificial fertilization. Hatching from the egg masses occurred 4-6 days after spawning at *ca.* 19°C. This developmental rate was approximately one day longer than that for *T. pacificus* paralarvae reared by artificial fertilization at the same temperature (Sakurai *et al.,* 1996). O'Dor *et al.* (1982b) also reported delayed hatching from *Illex illecebrosus* egg masses. The longer developmental period within egg masses suggests that animals reared by artificial fertilization might hatch at a premature stage. Watanabe *et al.* (1996) confirmed that artificially fertilized *T. pacificus* eggs hatched approximately two developmental stages earlier than eggs within the egg masses (stage criteria defined by Watanabe). The enveloping oviducal-gland and nidamental-gland jellies of the egg masses presumably reduce mechanical stimulation of developing embryos, a cause of premature hatching in some cephalopods (Choe, 1966).

Paralarvae

Durward *et al.* (1980) suggested that ommastrephid paralarvae might feed on microorganisms and plankton that colonize the egg mass. We saw no evidence of any feeding by the paralarvae within the egg mass after hatching, however the longer developmental time within the egg mass indicates greater opportunity for developing embryos to absorb organics from the oviducal gland jelly.

Hatching paralarvae swim upward immediately, with many animals found concentrated at the surface. Much biological emphasis has been placed on the surface film of organic matter in the sea (*e. g.* Sieburth *et al.*, 1976). Dissolved and particulate organic matter concentrations are significantly higher in the thin layer at the sea surface than in bulk seawater (Liss, 1975; Hunter and Liss, 1981). The possibility that cephalopods can use dissolved organic matter as a nutrition source has been proposed by Hanlon *et al.* (1991). Several studies have published evidence in support of this hypothesis (Castille and Lawrence, 1978; Vecchione and Hand, 1989). Further investigation of uptake of dissolved organics is needed, especially in the case of ommastrephid paralarvae.

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LITERATURE CITED

Akimushkin, I. I. 1963. [Cephalopods of the seas of the U. S. S. R. Nauka, Moscow. 236 pp. [In Russian] (English translation by A. Mercado, 1965, Israel Program for Scientific Translation, Jerusalem, 223 pp.)

Araya, H. 1976. Migration and fishing ground of winter sub-population of the squid, *Todarodes pacificus* Steenstrup, in the northern waters of Japan. *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 41:119-129.

Boletzky, S., R. Lowe, and L. Aroles. 1973. Spawning and development of the eggs, in the laboratory, of *Illex coindetii* (Mollusca, Cephalopoda). *The Veliger* 15:257-258.

Bradbury, H. E. and F. A. Aldrich. 1969. Observations on locomotion of the short-finned squid, *Illex illecebrosus* (LeSueur, 1821) in captivity. *Canadian Journal of Zoology* 47(5):741-744.

Castille, F. L. and A. L. Lawrence. 1978. Uptake of amino acids and hexoses from sea water by octopod hatchlings (abstract). *The Physiologist* 21:18.

Choe, S. 1966. On the eggs, rearing, habits of the fry, and growth of some Cephalopoda. *Bulletin of Marine Science* 16(2):330-348.

Durward, R. D., E. Vessey, R. K. O'Dor, and T. Amaratunga. 1980. Reproduction in the squid, *Illex illecebrosus*: first observations in captivity and implications for the life cycle. *International Commission for the Northwest Atlantic Fisheries Selected Papers* 6:7-13.

Hamabe, M. 1961a. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. 2. Spawning behavior.] *Zoological Magazine* 70(11):385-394. [In Japanese with English summary].

Hamabe, M. 1961b. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. 3. Early embryonic development and morphlogical [*sic*] features of the larvae immediately after hatching.] *Zoological Magazine* 70(11):408-419. [In Japanese with English summary].

Hamabe, M. 1963. [Spawning experiments of the common squid, *Ommastrephes sloani pacificus* Steenstrup, in an indoor aquarium.] *Bulletin of the Japanese Society of Scientific Fisheries* 29(10):930-934. [In Japanese with English summary].

Hanlon, R. T., P. E. Turk, and P. G. Lee. 1991. Squid and cuttlefish mariculture: an updated perspective. *Journal of Cephalopod Biology* 2(1):31-40.

Hunter, K. A. and P. S. Liss. 1981. Organic sea surface films. *In*: *Marine Organic Chemistry*, E. K. Duursma and R. Dawson, eds. pp. 259-298. Elsevier Scientific, Amsterdam.

Ikeda, Y., Y. Sakurai, and K. Shimazaki. 1993. Maturation process of the Japanese common squid *Todarodes pacificus* in captivity. *In*: *Recent Advances in Cephalopod Fisheries Biology*, T. Okutani, R. K. O'Dor, and T. Kubodera, eds. pp. 179-187. Tokai University Press, Tokyo.

Lapitkhovsky, V.V. and S. A. Murzov. 1990. [Epipelagic egg mass of the squid *Sthenoteuthis pteropus* collected in the tropical eastern Atlantic.] *Biologiya Morya* 3:62-63. [In Russian with English summary].

Liss, P. S. 1975. The chemistry of the sea surface microlayer. *In*: *Chemical Oceanography, Vol. 2, 2nd ed.*, J. P. Riley and G. Skirrow, eds. pp. 193-243. Academic Press, London.

Murata, M. 1978. [The relation between mantle length and body weight of the squid, *Todarodes pacificus* Steenstrup.] *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 43:33-51. [In Japanese with English summary].

Murata, M. 1989. Population assessment, management and fishery forecasting for the Japanese common squid, *Todarodes pacificus*. *In*: *Marine Invertebrate Fisheries: Their Assessment and Management*, J. R. Caddy, ed. pp. 613-636. John Wiley and Sons, New York.

Murata, M. 1990. Oceanic resources of squids. *Marine Behavior and Physiology* 18:19-71.

Murata, M., Y. Onoda, M. Tashiro, and Y. Yamagishi. 1971. [Ecological studies on the squid, *Todarodes pacificus* Steenstrup in the northern waters of the Japan Sea in 1970.] *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 37:10-31. [In Japanese with English summary].

Murata, M., Y. Onoda, M. Tashiro, Y. Yamagishi, and T. Suzuki. 1973. [Ecological studies on the squid, *Todarodes pacificus* Steenstrup in the northern waters of the Japan Sea in 1971.] *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 39:1-25. [In Japanese with English summary].

O'Dor, R. K. and N. Balch. 1985. Properties of *Illex illecebrosus* egg masses potentially influencing larval oceanographic distribution. *Northwest Atlantic Fisheries Organization Scientific Council Studies* 9:69-76.

O'Dor, R. K., N. Balch, and T. Amaratunga. 1982a. Laboratory observations of midwater spawning by *Illex illecebrosus*. *Northwest Atlantic Fisheries Organization Scientific Council Report* Document 82/VI/5, 8 pp.

O'Dor, R. K., E. Vessey, and T. Amaratunga. 1980. Factors affecting fecundity and larval distribution in the squid, *Illex illecebrosus*. *Northwest Atlantic Fisheries Organization Scientific Council*

Report Document No. 80/II/39, Ser. No. N070, 9 pp.

O'Dor, R. K., N. Balch, E. A. Foy, R. W. M. Hirtle, D. A. Johnston, and T. Amaratunga. 1982b. Embryonic development of the squid, *Illex illecebrosus*, and effect of temperature on development rates. *Journal of Northwest Atlantic Fisheries Science* 3(1):41-45.

Okutani, T. 1983. *Todarodes pacificus. In: Cephalopod Life Cycles, Vol. 1,* P. R. Boyle, ed. pp. 201-214. Academic Press, London.

Sabirov, R. M. , A. I. Arkhipkin, V. Y. Tsygankov, and A. S. Schetinnikov. 1987. [Egg laying and embryonal development of diamond-shaped squid, *Thysanoteuthis rhombus* (Oegopsida, Thysanoteuthidae).] *Zoologichesky Zhurnal* 66(8):1155-1163. [In Russian with English summary].

Sakurai, Y., Y. Ikeda, M. Shimizu, and K. Shimazaki. 1993. Feeding and growth of captive adult Japanese common squid, *Todarodes pacificus*, measuring initial body size by cold anesthesia. *In: Recent Advances in Cephalopod Fisheries Biology,* T. Okutani, R. K. O'Dor, and T. Kuboder, eds. pp. 467-476. Tokai University Press, Tokyo.

Sakurai, Y., J. R. Bower, Y. Nakamura, S. Yamamoto, and K. Watanabe. 1996. Effect of temperature on development and survival of *Todarodes pacificus* embryos and paralarvae. *American Malacological Bulletin* 13(1/2):89-95.

Sieburth, J. M., P. J. Willis, K. M. Johnson, C. M. Burney, D. M. Lavoie, K. R. Hinga, D. A. Caron, F. W. French, P. W. Johnson, and P. G. Davis. 1976. Dissolved organic matter and heterotrophic microneuston in the surface microlayers of the North Atlantic. *Science* 194:1415-1418.

Soeda, J. 1956. [Studies on the ecology and the breeding habits of the squid, *Ommastrephes sloani pacificus* (Steenstrup).] *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 14:1-24. [In Japanese with English summary].

Tashiro, M., Y. Yamagishi, and T. Suzuki. 1972. [Results of the tagging experiments of a common squidfish [*sic*] *Todarodes pacificus* (Steenstrup) made in off-shore area of the northern Japan Sea during a summer season of 1970. An attempt to grouping of the tagged squids by size.] *Scientific Report of the Hokkaido Fisheries Experimental Station* 14:1-16. [In Japanese with English summary].

Vecchione, M. and V. A. Hand. 1989. Digestive-gland histology in paralarval squids (Cephalopoda: Loliginidae). *Fishery Bulletin* 87:995-1000.

Vecchione, M. and C. F. E. Roper. 1991. Cephalopods observed from submersibles in the western North Atlantic. *Bulletin of Marine Science* 49(1-2):433-445.

Watanabe, K., Y. Sakurai, S. Segawa, and T. Okutani. 1996. Development of the ommastrephid squid *Todarodes pacificus*, from fertilized egg to rhynchoteuthion paralarva. *American Malacological Bulletin* 13(1/2):73-88.

Young, R. E. 1995. Aspects of the natural history of pelagic cephalopods of the Hawaiian mesopelagic-boundary region. *Pacific Science* 49(2):143-155.

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Development of the ommastrephid squid *Todarodes pacificus*, from fertilized egg to rhynchoteuthion paralarva

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Abstract: The present study establishes for the first time an atlas for the normal development of *Todarodes pacificus* Steenstrup, 1880, from fertilized egg to rhynchoteuthion paralarva. In the course of the study, observations on embryogenesis and histological differentiation in *T. pacificus* were made for consideration of the developmental mode of the Oegopsida, which is a specialized group with a reduced external yolk sac. It appears that differentiation of the respiratory and digestive organs is relatively delayed in the Oegopsida, with reduction of the yolk sac as well as the egg size. These characters could be related to a reproductive strategy for paralarval dispersion in the open ocean.

Key words: *Todarodes pacificus*, oceanic squid, development, rhynchoteuthion, yolk sac

The pelagic squid, *Todarodes pacificus* Steenstrup, 1880, is distributed all around Japan and its neighboring waters, extending from the northern part of the Kurile Islands south to Hong Kong (Okutani *et al.*, 1987; Okutani, 1995). This squid is one of the most commercially important cephalopods in Japan. Knowledge of the early life history of *T. pacificus* would provide basic information for fishery biology. It would also be invaluable in the analysis of the phylogeny of the Cephalopoda by clarifying the embryogenesis of an oegopsid species that has seldom been previously pursued by researchers.

There are only a few embryological studies on the Oegopsida, despite the many embryological studies on the Myopsida (*e. g.* Naef, 1928; Arnold, 1965, 1990; Segawa, 1987; Segawa *et al.*, 1988; Baeg *et al.*, 1992) and Sepioidea (*e. g.* Naef, 1928; Yamamoto, 1982). Soeda (1952, 1954) and H. Hayashi (1960) observed the embryonic development of *Todarodes pacificus* from artificially inseminated eggs, and described only a part of the development because embryonic development became abnormally arrested. Hamabe (1961, 1962) described and illustrated the embryonic development of *T. pacificus* without defining developmental stages. Naef (1928) proposed the developmental stages for one ommastrephid species (as ommastrephid Y), subsequently identified as *Illex coindetii* (Verany, 1837) (Boletzky *et al.*, 1973), but the stages were separated by large intervals and applied only after organogenesis. Recent observations of the embryonic development of *I. illecebrosus* were made by O'Dor *et al.* (1982) without providing an atlas of stages.

The development of a technique of artificial fertilization for ommastrephid squids (Sakurai and Ikeda, 1992; Ikeda *et al.*, 1993; Sakurai *et al.*, 1995) has made it possible to examine the embryonic development and early life stages of *Todarodes pacificus*. In the present study, embryogenesis and histological differentiation of *T. pacificus* were observed in artificially fertilized eggs, and a complete atlas of developmental stages was established for the first time. Comparisons are made with several other species of the Oegopsida (Ommastrephidae, Enoploteuthidae, and Thysanoteuthidae), Myopsida, and Sepioida (Watanabe, unpub.) to characterize the developmental mode of oceanic oegopsids.

MATERIAL AND METHODS

Artificial Fertilization and Cultivation

Fertilized eggs of *Todarodes pacificus* were obtained by artificial fertilization using the method described by Sakurai *et al.* (1995). Female squids were captured in the waters near Hakodate, Hokkaido, and maintained in a tank at the Usujiri Fisheries Laboratory of Hokkaido University until they reached maturity. Ova were obtained by dissecting the oviducts of a sacrificed female. Sperm masses were dissected from the seminal receptacles in the labral area of the same female and suspended in filter-sterilized, aerated seawater. After the sperm-seawater mixture was added to the ova, freeze-dried oviducal gland powder, which had been dissolved in seawater beforehand, was added and mixed with the ova in a petri dish. The eggs were divided into groups of 15-20 per petri dish (60 mm in diameter) filled with filter-sterilized, aerated seawater. The seawater in each petri dish was changed twice daily. The fertilized eggs were incubated at 17°, 20°, and 23°C, on the advice of Sakurai *et al.* (1996) who reported that the peak of embryonic survival rates occurred between 14.7° and 22.2°C. At each temperature unit, about 1,000 eggs were incubated for morphological and histological observations.

Observation of Development and Determination of Stages

Observations were made of developing embryos under a light microscope and an atlas of developmental stages was prepared. Regular stages were identified mainly from embryos incubated at 20°C and supplementary observations at either 17° or 23°C were made for stages 9, 10, 11, 19, 20, 21, and 25.

To discriminate the developmental stages, the criteria established by Naef (1928) for ommastrephid Y and Arnold (1965, 1990) for *Loligo* were used. In the following description in the Results section, Arabic stage numerals in brackets represent the stages of Arnold (1965, 1990) and Roman stage numerals represent the stages of Naef (1928).

Developmental stages were defined to seven days after hatching, because many organs were differentiated after hatching. Measurements and means were taken on at least ten live animals.

Observation of Hatchlings from an Egg Mass

In addition to artificially fertilized eggs, newly emerged hatchlings from an egg mass spawned in a tank at the Usujiri Fisheries Laboratory of Hokkaido University on 30 September 1994 (Bower and Sakurai, 1996) were investigated. Eggs within the large spherical egg mass were surrounded by a large quantity of nidamental and oviducal gland jelly.

Histological Observation

Histological sections were prepared by fixing the specimens in Bouin's fixative, embedding in paraffin, sectioning, and staining with haematoxylin and eosin.

RESULTS

ATLAS OF DEVELOPMENT

Ova were ovoid in shape, and measured *ca.* 0.83 x 0.70 mm before fertilization. Following fertilization, the egg shape became spherical, measuring *ca.* 0.74 mm in diameter. The embryos took 90-95 hr at 20°C to develop from fertilization to hatching *in vitro* and the mean mantle length of hatchlings was 0.95 mm.

After hatching, paralarvae were maintained for up to *ca.* seven days without being fed while the internal yolk was completely absorbed. Paralarval mantle length measured 1.25 mm on the seventh day. The proboscis started to grow on the second day after hatching. The length of the proboscis from the anterior edge of the eyes was 0.27 mm on the third day and 0.49 mm on the seventh day, respectively. Arms III were not yet differentiated seven days after hatching.

In artificially fertilized eggs, hatching occured at stage 26, whereas eggs that developed within the egg mass spawned in the tank hatched at stage 28.

Fertilization and Meiosis (Figs. 1 and 5)

Stage 1 (Stage 1 of Arnold), 10 min after insemination: Fertilization. Following fertilization, perivitelline space expands and egg shape changes from ovoid to spherical. Micropyle is visible at the animal pole.

Stage 2 (Stage 2), 30 min: First maturation division. First polar body appears close to the animal pole.

Stage 3 (Stage 3), 1.5 hr: Second maturation division. Second polar body appears next to the first. Polar bodies are visible until stages 12 or 13. Blastodisc appears and increases in size. The blastodisc area is more transparent than the ooplasmic area.

Cleavage (Figs. 1 and 5)

Stage 4 (Stage 4), 2.6 hr: First cleavage. The furrow occurs at the center of the blastodisc running beneath the polar bodies and extending to the equator of the egg.

Stage 5 (Stage 5), 3.7 hr: Second cleavage. Second furrow occurs across the first one at right angle and divides the first cells into anteriorly, where the polar bodies are situated, and posteriorly located segments.

Stage 6 (Stage 6), 4.5 hr: Third cleavage. The cells are divided differently in the anterior half and the posterior half. In the posterior half, the furrow slants, almost parallel to the first one, while in the anterior half it extends radially pulling the second one forward.

Stage 7 (Stage 7), 5.0 hr: Fourth cleavage. Inner four cells are established as blastomeres while the surrounding 12 cells remain contiguous at their outer margins as blastocones.

Stage 8 (Stage 8), 5.5 hr: Fifth cleavage; 32 cells (14 blastomeres and 18 blastocones).

Stage 9 (Stage 9), 4.5 hr at 23°C: Sixth cleavage. The cells divide asynchronously and finally become 64 cells. A group of eight very small cells exists near the cen-

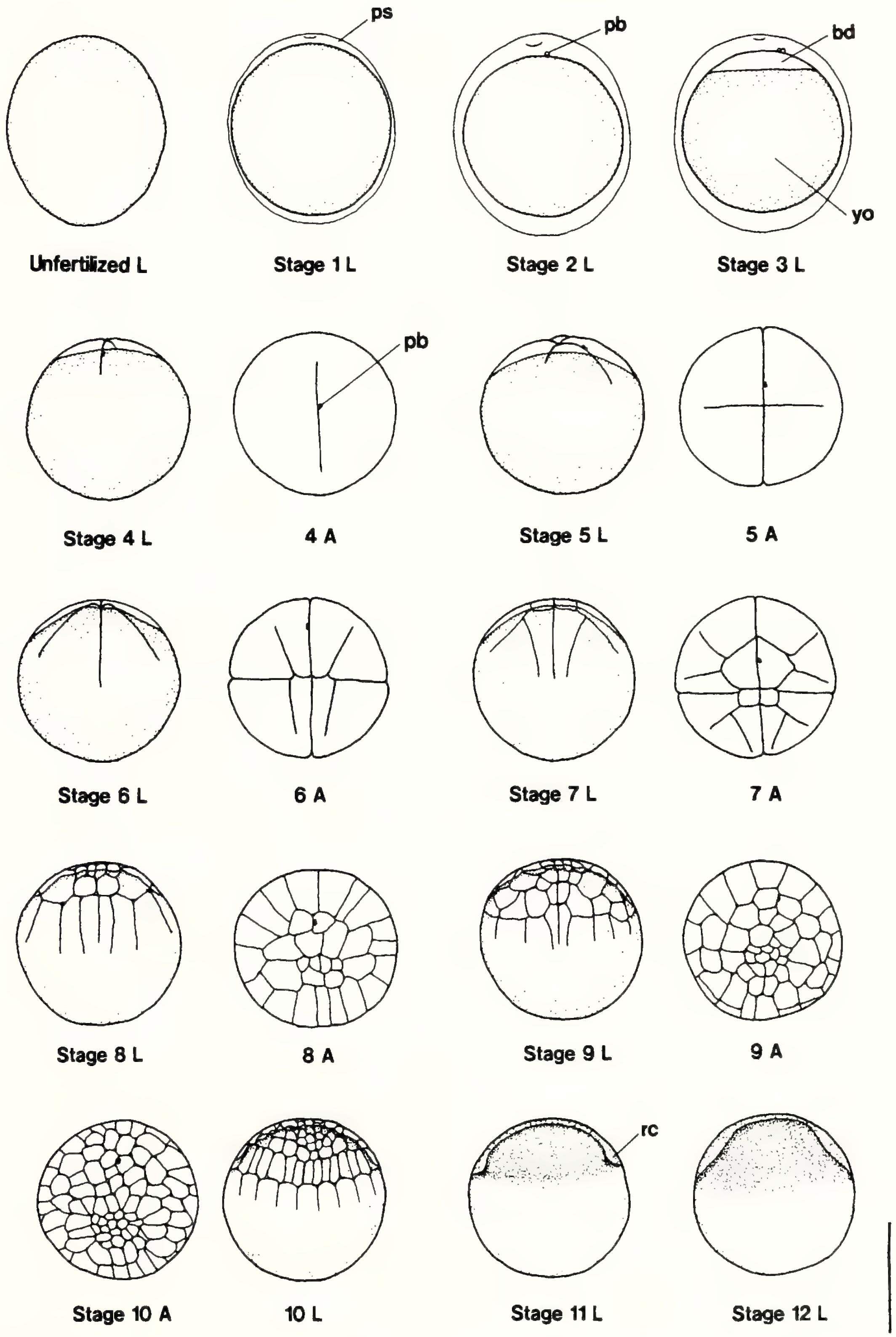
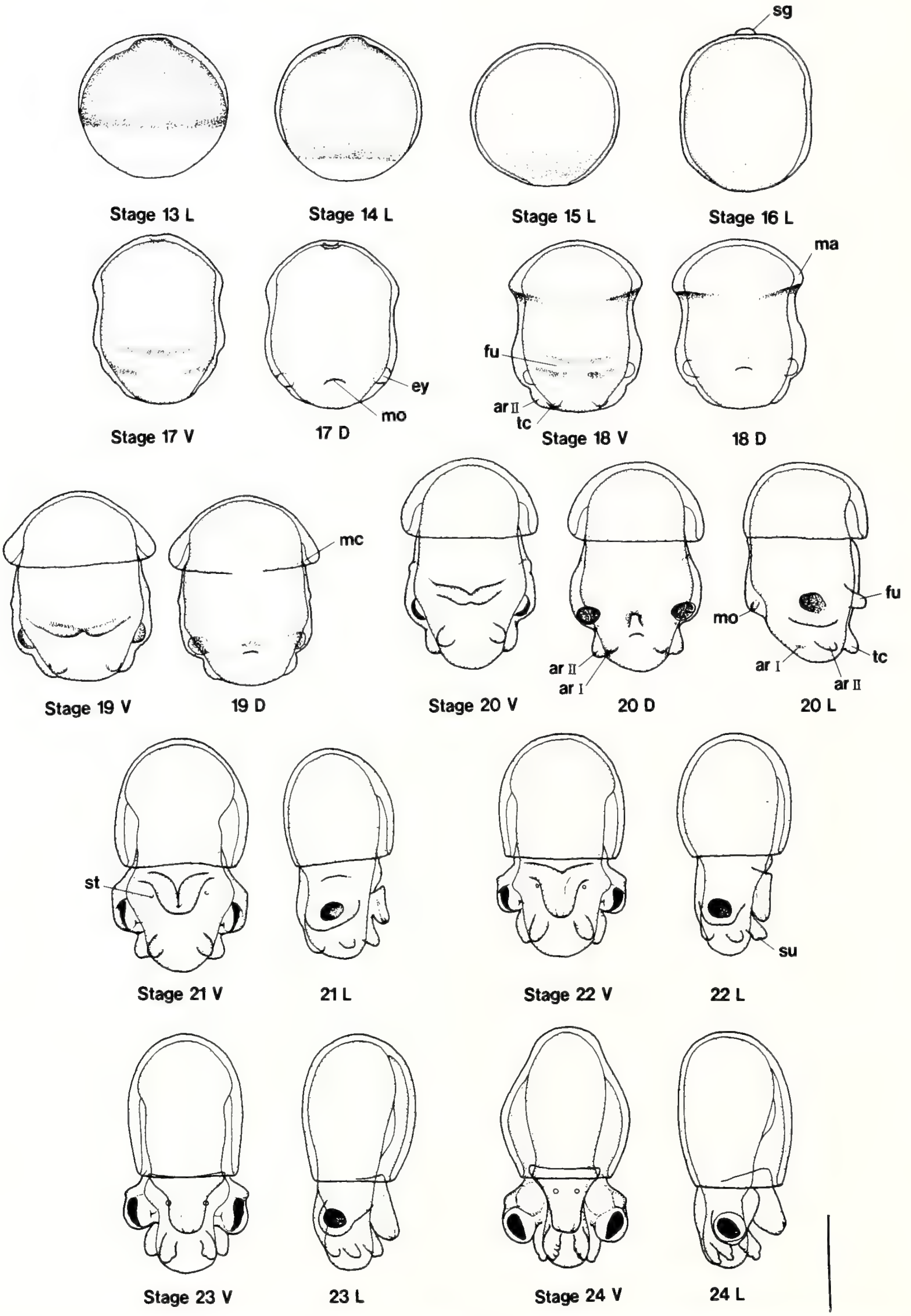

ps
pb
bd
yo
Unfertilized L
Stage 1 L
Stage 2 L
Stage 3 L
pb
Stage 4 L
4 A
Stage 5 L
5 A
Stage 6 L
6 A
Stage 7 L
7 A
Stage 8 L
8 A
Stage 9 L
9 A
rc
Stage 10 A
10 L
Stage 11 L
Stage 12 L

Fig. 1. Development of *Todarodes pacificus*, from fertilized egg to Stage 12 embryo. A or L with stage number indicates apical or lateral view. Egg membrane is omitted from Stage 4. Scale bar = 0.5 mm. (bd, blastodisc; pb, polar body; ps, perivitelline space; rc, ring-shaped group of cells; yo, yolk).


sg
Stage 13 L
Stage 14 L
Stage 15 L
Stage 16 L
ma
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fu
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ar II
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Stage 17 V
17 D
Stage 18 V
18 D
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fu
mo
tc
ar II
ar I
ar I
ar II
Stage 19 V
19 D
Stage 20 V
20 D
20 L
st
su
Stage 21 V
21 L
Stage 22 V
22 L
Stage 23 V
23 L
Stage 24 V
24 L

Fig. 2. Development of *Todarodes pacificus*, from Stage 13 to Stage 24 embryo. D, L, or V with stage number indicates dorsal, lateral, or ventral view, respectively. Egg membrane is omitted. Scale bar = 0.5 mm. (ar, arm; ey, eye; fu, funnel; ma, mantle; mc, mantle cavity; mo, mouth; sg, shell gland; st, statocyst; su, sucker; tc, tentacle club).

ter; this was designated as the future shell gland by Arnold (1990).

Stage 10 (Stage 9+), 5.0 hr at 23°C: Cleavage continues asynchronously. The group of very small cells remains recognizable centrally.

Segregation of the Germ Layers and Growth of Blastoderm (Figs. 1, 2, and 5)

Stage 11 (Stage 10), 5.8 hr at 23°C: The cell size becomes smaller and almost uniform. A ring-shaped group of cells appears around the yolk mass below the margin of the blastoderm, which is caused by marginal superposition of the single early layer of the germ layer, as described by Boletzky (1988) and Arnold (1990).

Stage 12 (Stages 11-15), 19.0 hr at 17°C: The inner layer spreads toward the animal pole below the outer layer. The yolk mass becomes papilla-like in the center of the blastoderm at the animal pole. The edge of the blastoderm expands toward the vegetal pole and covers almost half of the egg.

Stage 13 (Stages 11-15), 19.5 hr: The blastoderm covers approximately two-thirds of the egg. The papilla-like yolk apex gradually becomes smaller with the formation of the inner germ layer.

Stage 14 (Stages 11-15), 26.5 hr: The blastoderm covers approximately three-quarters of the egg.

Stage 15 (Stages 11-15), 33.0 hr: The inner layer of the blastoderm closes at the vegetal pole and the papilla-like yolk apex disappears. Both layers of blastoderm nearly (but not quite) close at the vegetal pole.

Organogenesis (Figs. 2, 3, 5, 6, and 8)

Stage 16 (Stage 16), 42 hr: The embryo expands vertically. Major organ primordia appear as thickenings, with the primordium of the shell gland especially evident as a projection.

Stage 17 (Stage VIII of Naef), 50 hr: Invaginations of the shell gland and the mouth begin. The thickenings of the major organs progress. Primordia of eyes are visible.

Stage 18 (Stage VIII+), 54 hr: Elevation of mantle, funnel, eyes, tentacle clubs, and arms II begins. The shell gland is closed.

Stage 19 (Stage X), 40 hr at 23°C: Formation of mantle margins begins at ventral side. The funnel folds on each side rise clearly, but fusion in the midline has not yet commenced. Primordia of tentacle clubs and arms II are clearly visible.

Stage 20 (Stage X+), 43 hr at 23°C: Faint primordia of arms I appear. Retina pigmentation begins. Mantle cavity spreads into dorsum. Anterior parts of the funnel folds unite by margin. A row of faint chromatophores is first visible on the ventral and dorsal mantle margins. Embryo revolves around the vertical axis.

Stage 21 (Stage X++), 46 hr at 23°C: Funnel folds are fusing. Statocyst invagination begins. Optic stalks increase in height. Two rows of chromatophores are visible on the anterior mantle surface.

Stage 22 (Stage XII), 68 hr: Funnel tube is established and is not covered by the mantle. First sucker primordia appear on the tentacle clubs. Three rows of chromatophores are visible on the mantle.

Stage 23 (Stage XII+), 72 hr: Mantle covers the posterior margin of the funnel. Four rows of chromatophores are visible on the mantle.

Stage 24 (Stage XIV), 78 hr: Cephalic organs, such as eyes and optic ganglia, are concentrated. Yolk is transferred from yolk sac of cephalic region to mantle cavity. Four sucker primordia on the tentacle clubs and one sucker primordium on each arm are clearly visible. Positions of the suckers are different on right and left tentacle clubs. The chromatophores on the mantle become larger and more distinct.

Stage 25 (Stage XIV+), 67 hr at 23°C: A gutter is formed at the posterior apex of inner yolk sac by esophagus. Lens primordia are first visible. Primordia of ventral organs, such as gills, branchial hearts, systemic heart, stomach, caecum, and anal knoll, become prominent as three swellings. Bases of the two tentacle clubs come together in the midline. Hatching can occur by external stimulation.

Stage 26 (Stage XVI), 92 hr: Hatching. Primary lids cover the eye vesicles.

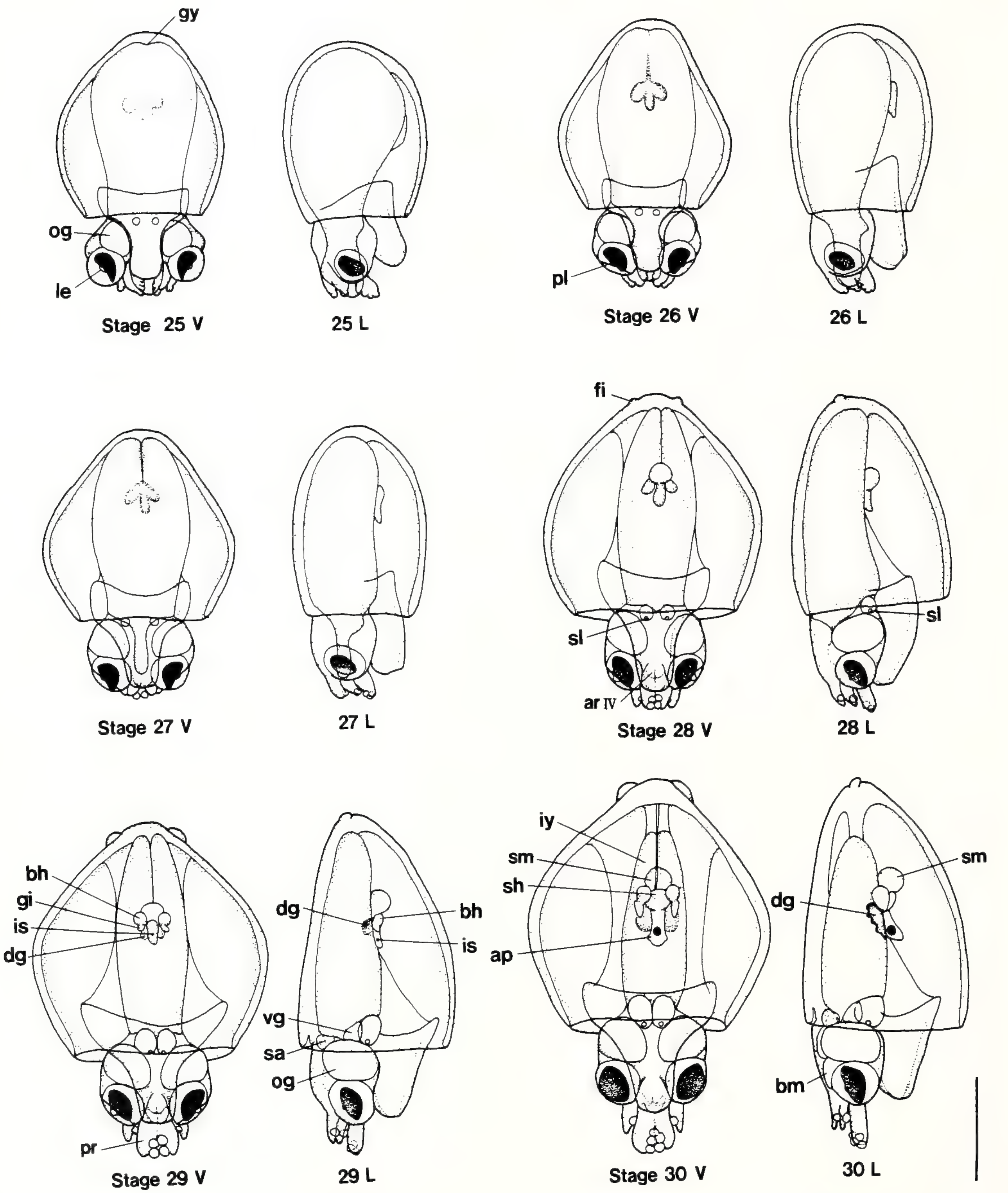
Post-Hatching (Figs. 3, 4, 7, and 8)

Stage 27 (Stage XVI+), 4 hr after hatching: Yolk sac at cephalic region is contracting. The gutter of the posterior apex of inner yolk sac deepens.

Stage 28 (Stage XVIII), 1 day after hatching: Yolk sac at cephalic region almost disappears. Fin primordia appear on the apex of the mantle. Primordia of arms IV are first visible. Statoliths are evident in both statocysts. Head chromatophores are first visible. Systemic heart is pulsating. Hatching from egg mass spawned in tank occurs at this stage.

Stage 29 (Stage XVIII+), 2 days after hatching: A stalk on the base of the tentacles begins to elongate, forming the so-called proboscis. The right and left club suckers, which were at different positions from stage 24 onwards, are combined together in a symmetrical pattern. Ink begins to be concentrated in ink sac. Primodia of gills and branchial hearts are evident. Digestive gland and salivary gland are visible.

Stage 30 (Stage XX), 3 days after hatching: Inner yolk sac has contracted and separated from the posterior end of the mantle. The eyes are finally covered by primary


gy
og
le
Stage 25 V
25 L
pl
Stage 26 V
26 L
Stage 27 V
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fi
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Stage 30 V
sm
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Fig. 3. Development of *Todarodes pacificus*, from Stage 25 embryo to Stage 30 rhynchoteuthion paralarva. L or V with stage number indicates lateral or ventral view. Egg membrane is omitted. Scale bar = 0.5 mm. (ap, anal papilla; ar, arm; bh, branchial heart; bm, buccal mass; dg, digestive gland; fi, fin; gi, gill; gy, gutter of inner yolk sac; is, ink sac; iy, inner yolk sac; le, lens, og, optic ganglion; pl, primary lid; pr, proboscis; sa, salivary gland; sh, systematic heart; sl, statolith; sm, stomach; vg, visceral ganglion).

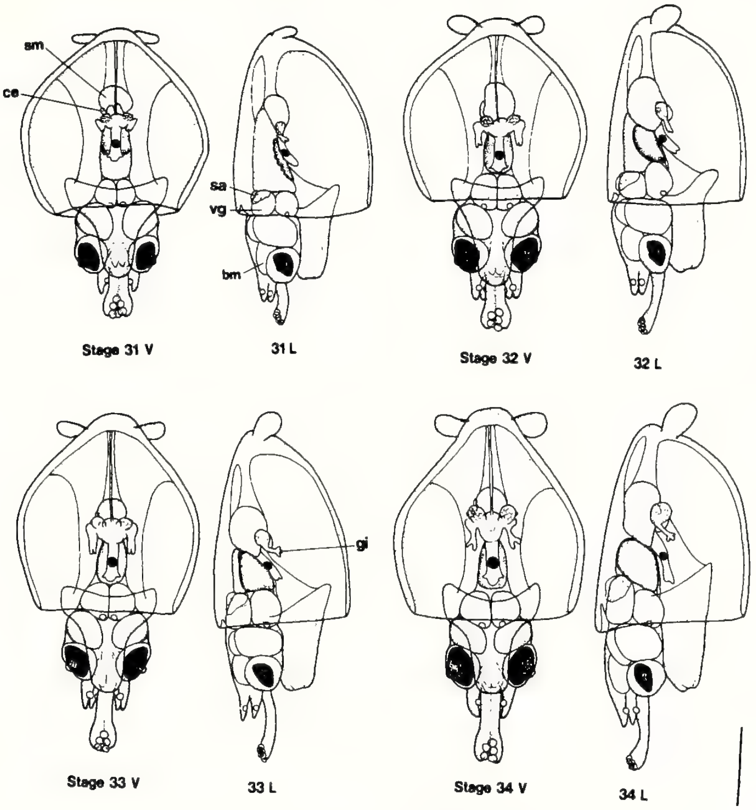

Fig. 4. Development of *Todarodes pacificus,* from Stage 31 to Stage 34 rhynchoteuthion paralarva. L or V with stage number indicates lateral or ventral view. Scale bar = 0.5 mm. (bm, buccal mass; ce, caecum; gi, gill; sa, salivary gland; sm, stomach; vg, visceral ganglion).

lid except protruded lens. Stomach is visible. Intestine grows and anal papillae are evident.

Stage 31, 4 days after hatching: Inner yolk sac becomes smaller and its posterior part lies behind the stomach in ventral view. Caecum is visible. Digestive gland grows larger. Ink sac is filled with ink. The stomach shows peristaltic movement. The suckers of the proboscis have primordia of chitinous rings.

Stage 32, 5 days after hatching: Posterior part of the inner yolk sac lies behind the systemic heart in ventral view. Digestive gland becomes larger. The proboscis stretches and contracts with the suckers moving.

Stage 33, 6 days after hatching: Branched gills are visible.

Stage 34, 7 days after hatching: Yolk almost consumed.

HISTOLOGICAL OBSERVATION

Histological observations of the digestive and respiratory organs revealed that they were still immature at the stage of hatching (Figs. 9A-B). From stage 28, the radula sac began to differentiate and started to secrete chitinous material (rs, Fig. 9C). Radula teeth were well developed and extended along the entire length of the ribbon by stage 29 (rs and rt, Fig. 9D). Upper and lower plates of the jaw also developed at stage 29 (uj and lj, Fig. 9D).

Rudiments of the digestive gland were histologically distinct from the surrounding mesoderm as a pair of monolayered saccular organs at stage 26, the lumina extending from the primary alimentary canal. From about stage 28, the stomach rudiment became distinct as a saccular body (sm, Fig. 9A). High vascularization in the digestive gland started at stage 29, and the lumen of the structure of the originally sac-like organ became complex. From stage 31, the caecum was differentiated, but the stomach and caecum were not clearly divided internally. The digestive duct appendages (the so-called pancreas) were histologically distinct from the digestive gland (the so-called liver) at stage 31. Differentiation of glandular cells in the digestive gland was conspicuous at stage 32. The space occupied by the digestive gland in adults was largely occupied by yolk in the hatchling. Fusion of the pair of rudimentary digestive glands began at stage 34 when the yolk was almost completely absorbed.

The rudiments of the salivary gland were histologi-

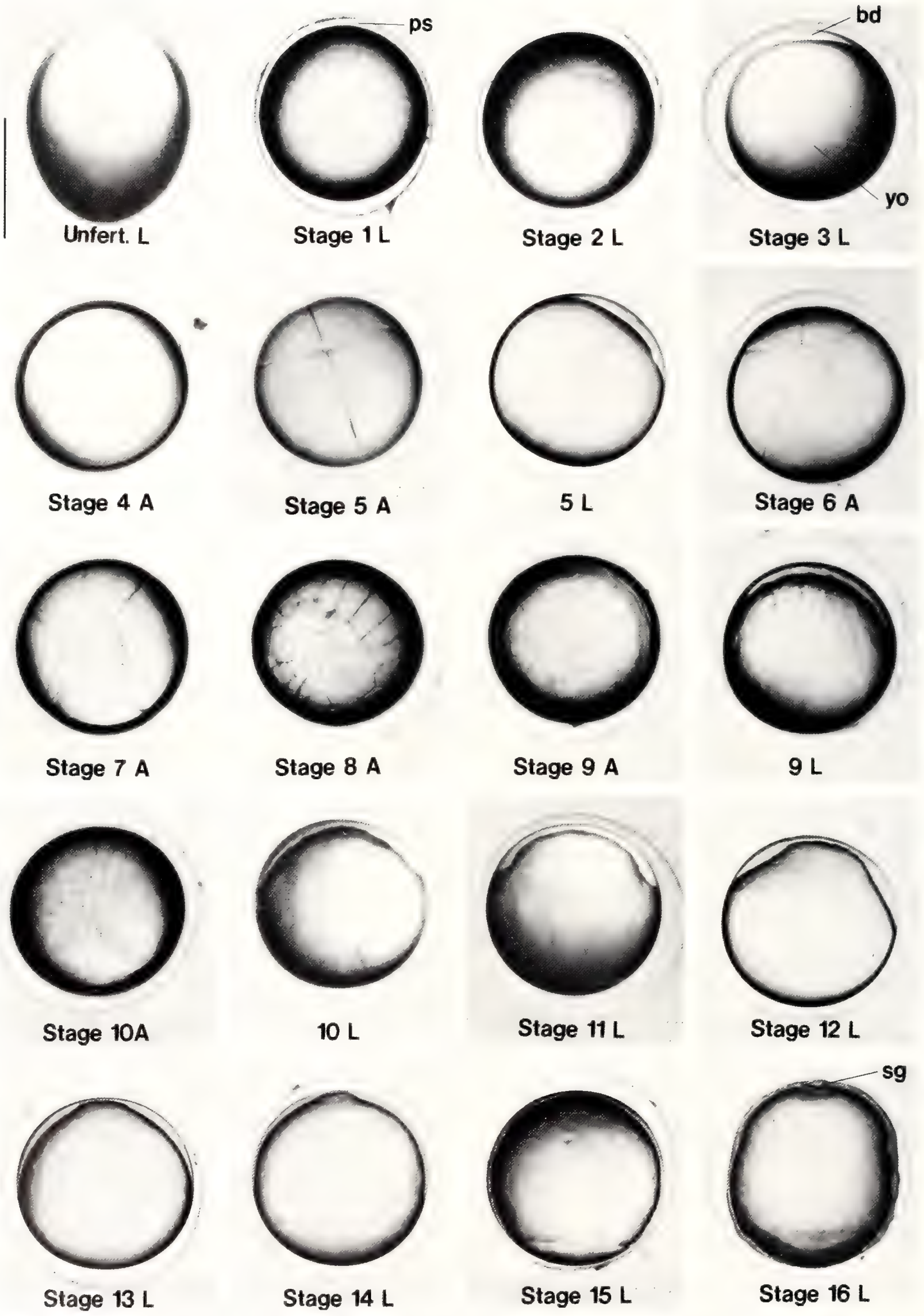
ps
bd
yo
Unfert. L
Stage 1 L
Stage 2 L
Stage 3 L
Stage 4 A
Stage 5 A
5 L
Stage 6 A
Stage 7 A
Stage 8 A
Stage 9 A
9 L
Stage 10A
10 L
Stage 11 L
Stage 12 L
sg
Stage 13 L
Stage 14 L
Stage 15 L
Stage 16 L

Fig. 5. Unfertilized egg and Stages 1-16 in the development of *Todarodes pacificus*. A or L with stage number indicates apical or lateral view. Scale bar = 0.5 mm. (bd, blastodisc; ps, perivitelline space; sg, shell gland; yo, yolk).

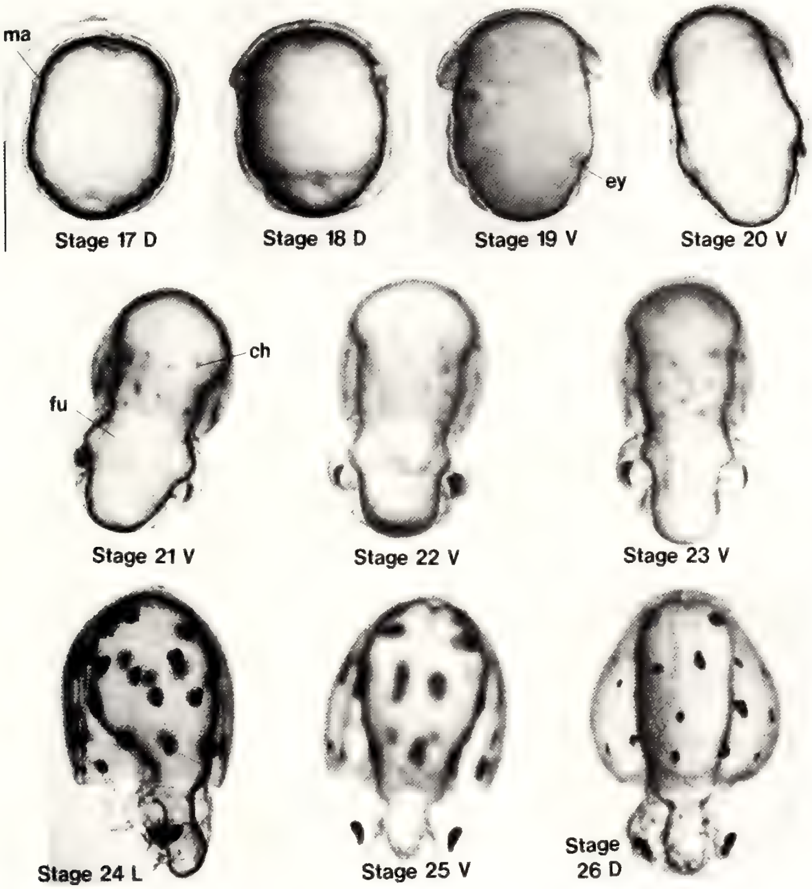

Fig. 6. Stages 17-26 in the development of *Todarodes pacificus.* D, L, or V with stage number indicates dorsal, lateral, or ventral view, respectively. Scale bar = 0.5 mm. (ch, chromatophore; ey, eye; fu, funnel; ma, mantle).

cally distinct as a monolayered sac-like papilla at stage 28 (sa, Fig. 9E). Glandular cells started to differentiate, and secretion of granules (stained by eosin) was observed in the lumen of salivary glands at around stage 32 (sa, Fig. 9F).

Todarodes pacificus has no distinct external yolk sac, but a dome-shaped external yolk sac was seen at late embryonic stages. No blood sinus was observed in the dome-shaped external yolk sac. The blood space, which includes primodia of the branchial and systemic hearts, and major vessels, such as the aorta posterior and vena cava, began to appear at stage 26. At the same time, the pericardial cavity developed.

The rudiments of gills became visible at stage 28 (gi, Fig. 10A), and lamellae of the gills became distinct at stage 30 (gi, Fig. 10B). The development of gills was so slow that only three rudimentary lamellae were observed at stage 34.

A functional Hoyle's organ became visible at stage 25 just before hatching (stage 26), and quickly degenerated at stage 27 just after hatching. In contrast, a functional, active Hoyle's organ was still visible at stage 28 in hatchlings taken from the egg mass.

DISCUSSION

For comparative studies on cephalopod development, common criteria of developmental stages must be established. Most previous embryological investigations (*e. g.* O'Dor *et al.*, 1982; Segawa *et al.*, 1988; Arnold and O'Dor, 1990; Baeg *et al.*, 1992) have been based either on the stages of Naef (1928) for *Sepia officinalis* Linné, 1758, *Loligo vulgaris* Lamarck, 1798, and ommastrephid Y, or on the stages of Arnold (1965) for *L. pealeii*. There is correspondence between Naef (1928) for *L. vulgaris* and Arnold (1965) for *L. pealeii* (Segawa *et al.*, 1988). It is, however, impossible for all of the stages of *Loligo* to be applied to *Todarodes pacificus*, due to differences in the sequence of differentiation of major features, such as gills, branchial hearts, caecum, and stomach (Fig. 11). Because Naef (1928) never provided any early developmental stages for

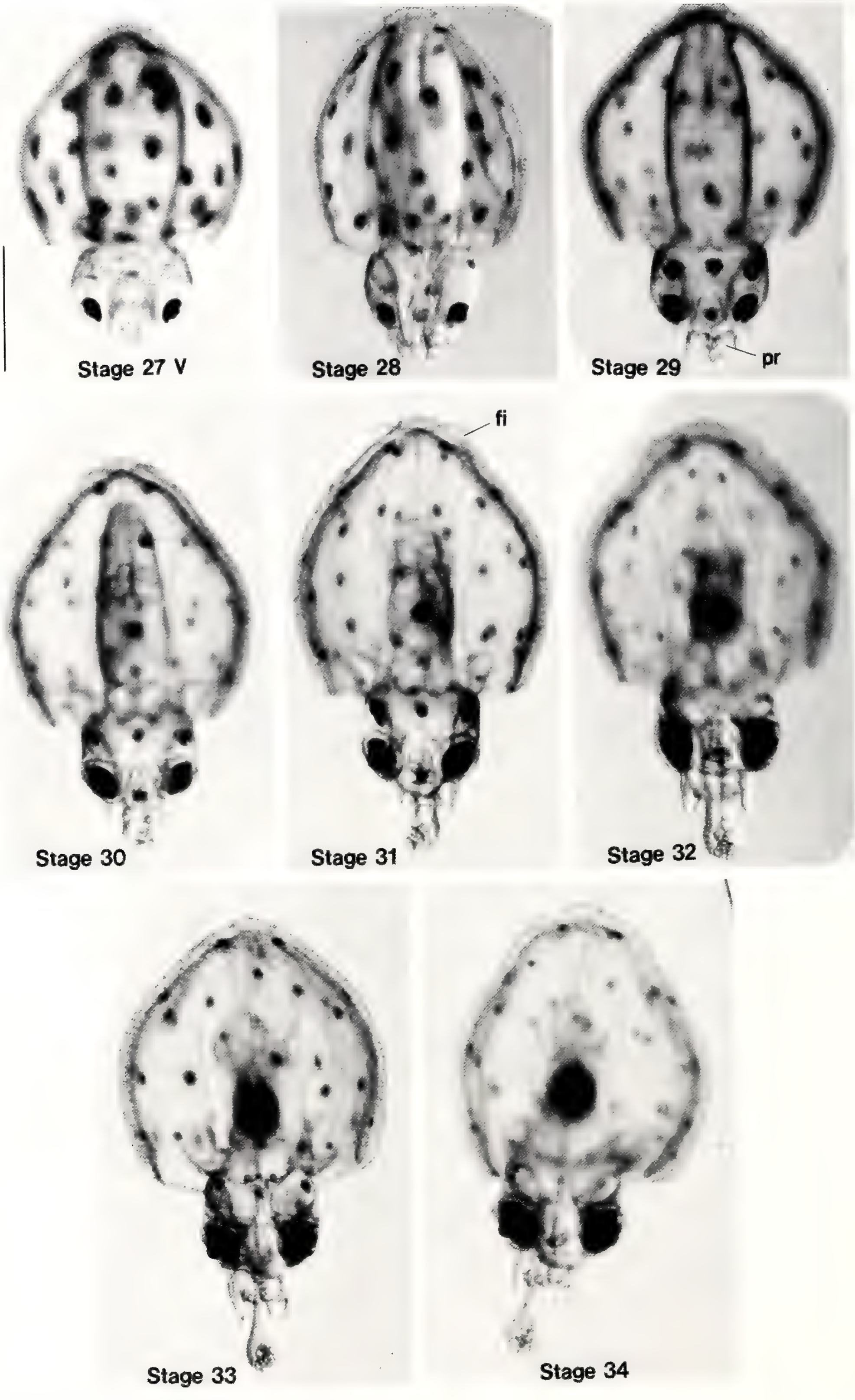
Stage 27 V
Stage 28
Stage 29
pr
fi
Stage 30
Stage 31
Stage 32
Stage 33
Stage 34

Fig. 7. Stages 27-34 in the development of *Todarodes pacificus,* ventral view. Scale bar = 0.5 mm. (fi, fin; pr, proboscis).

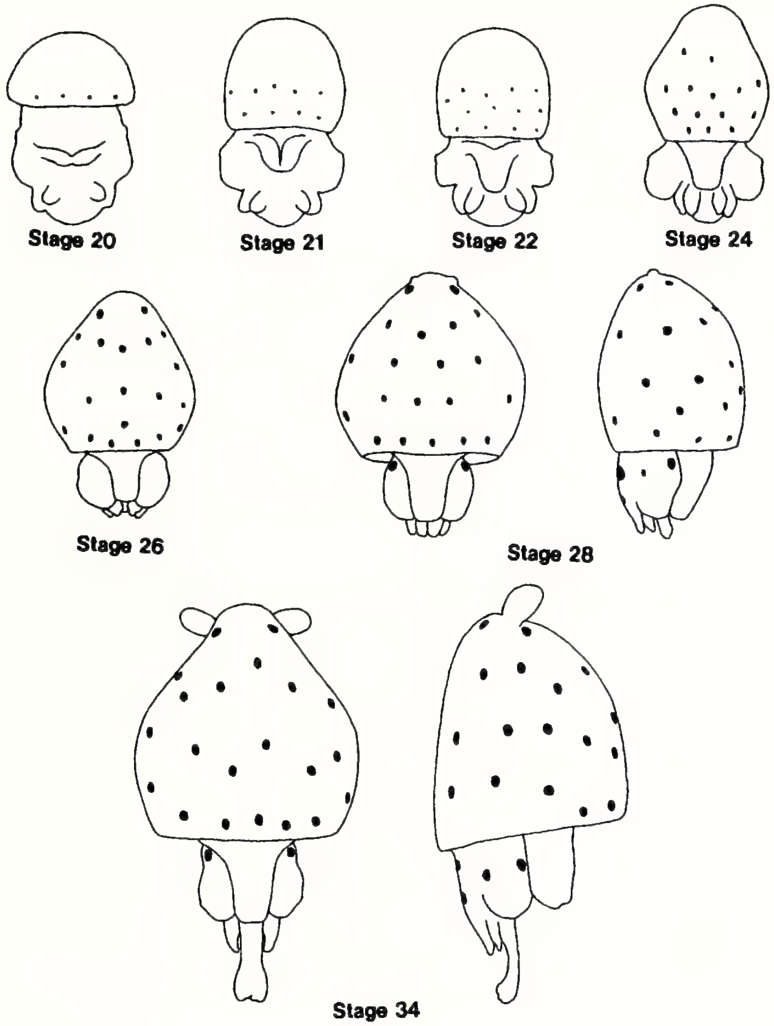

Fig. 8. Sequence of chromatophore development (Stages 20-34).

Ommastrephidae, the criteria of Arnold (1965) were used in the present study. The criteria for the phase of cleavage of *Loligo* (Arnold, 1965) are applicable to *T. pacificus,* although there are some differences in the blastoderm formation: the cleavage furrows reach the equator in eggs of *T. pacificus,* whereas the furrow length of *Loligo* represents only a small fraction of the egg perimeter.

There are differences between artificially fertilized eggs and eggs from the egg mass spawned in the tank. The eggs from the egg mass hatched approximately two stages later than artificially fertilized eggs. A key difference during development between artificially fertilized eggs and those from the egg mass in the present study was that the latter were enwrapped by nidamental and oviducal gland jelly. The observation of the development of Hoyle's organ suggests that hatching can occur between stages 25 and 28, and the timing of hatching can vary slightly, depending on external conditions of the eggs. It is possible that the presence of nidamental and oviducal gland jelly could somehow delay hatching.

In Naef's (1928) description of the developmental stages of an ommastrephid squid *(Illex coindetii)*, hatching occurs at stage XX. *I. illecebrosus* observed by O'Dor *et al.* (1982) reportedly hatched at the same stage XX of Naef. However, *Todarodes pacificus* hatched at stage XVI from artificially fertilized eggs and at stage XVIII from the egg mass. The newly hatched *T. pacificus* described by Hamabe (1962) appeared to be at stages XIV or XVI although he did not define any developmental stages. It is not certain whether this difference in hatching stage is due to a difference among species or to an environmental factor.

Hamabe (1962) assumed that arms III appear earlier than arms IV, based on an observation on hatchlings two days old (1.1 mm mantle length [ML]) and planktonic par-

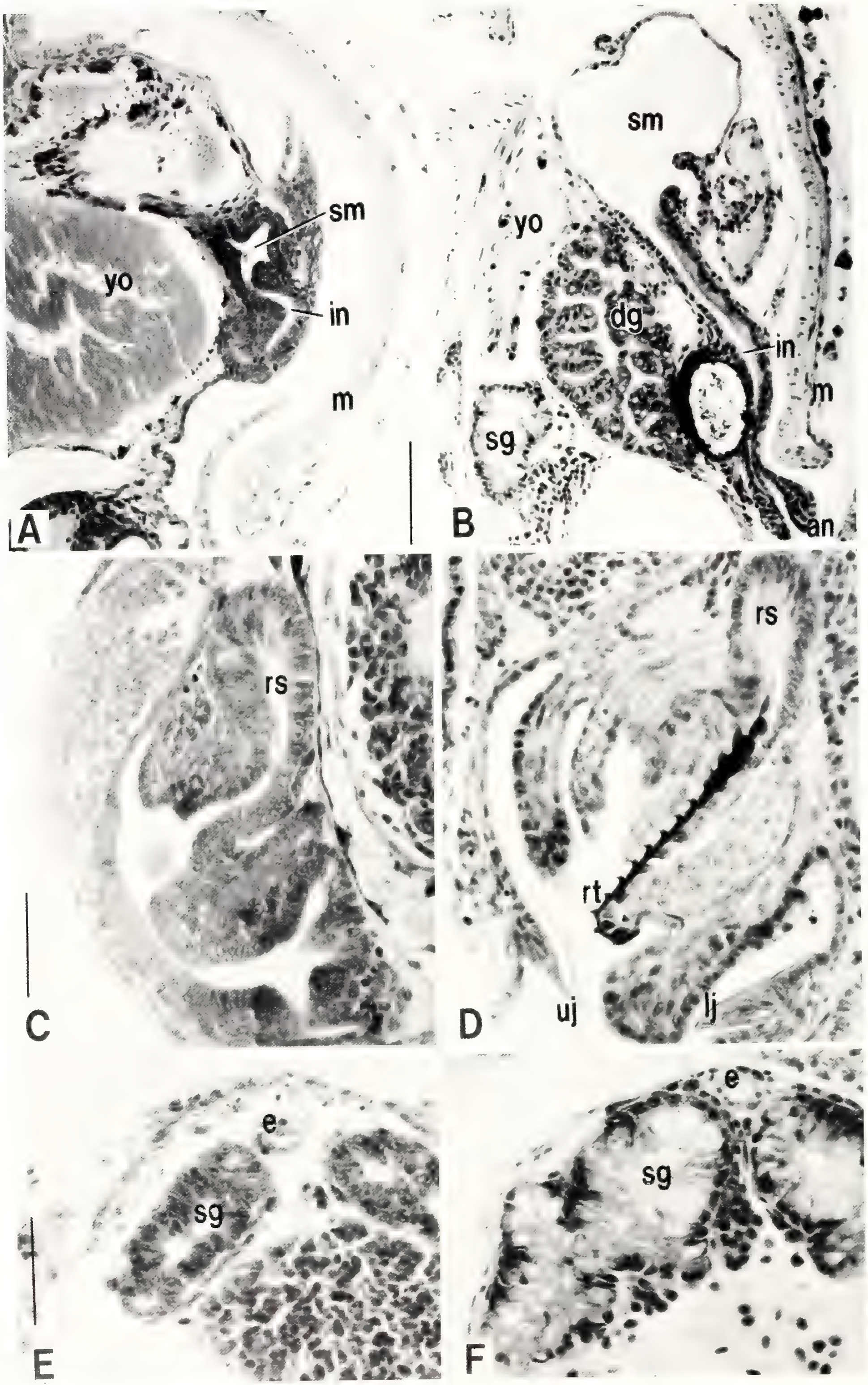

Fig. 9. Histological differentiation of digestive organs of *Todarodes pacificus* around hatching stage. A-B. Alimentary canal, longitudinal sections at Stages 28 (A) and 32 (B). C-D. Buccal mass, longitudinal sections at Stages 29 (C) and 31 (D). E-F. Salivary gland, cross-sections at Stages 30 (E) and 32 (F). Scale bars = 100 μm (A-B); 50 μm (C-F). (an, anus; dg, digestive gland (so-called liver); e, esophagus; in, intestine; lj, lower jaw; m, mantle; rs, radula sac; rt, radula teeth; sg, salivary gland; sm, stomach; uj, upper jaw; yo, yolk).

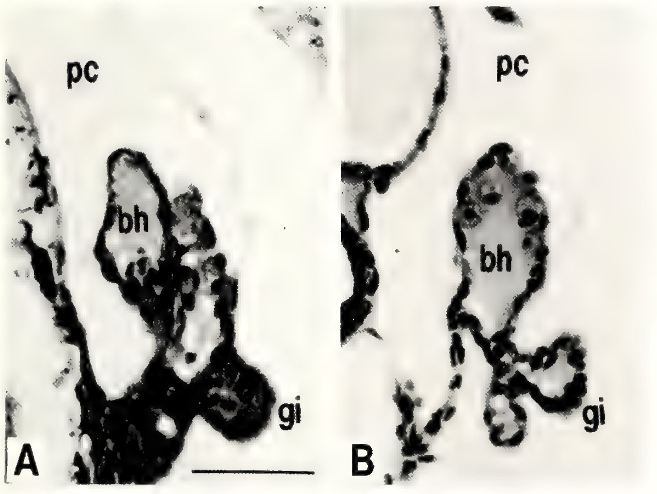

Fig. 10. Histological differentiation of the gills of *Todarodes pacificus*, longitudinal sections at Stages 28 (A) and 31 (B). Scale bar = 50 µm. (bh, branchial heart; gi, gill; pc, pericardial cavity).

alarvae (3-5 mm ML) captured from the sea. Hamabe could have mistaken arms IV for arms III, due to lack of information on intermediate specimens between the hatchling (1 mm ML) and later stage paralarvae (3 mm ML). The arms III appear and increase in size rapidly and outgrow arms IV, I, and II, while arms IV grow very slowly.

In *Todarodes pacificus*, the differentiation of digestive and respiratory organs is postponed until posthatching stages (Fig. 11). A similar tendency has been observed in other oegopsid squids, such as *Abraliopsis* sp. (see Arnold and O'Dor, 1990), *Illex illecebrosus* (see O'Dor *et al.*, 1982), and *Watasenia scintillans* (see S. Hayashi, 1995). In the Sepioidea and Myopsida, development of the digestive organs is almost completed long before hatching (Naef, 1928), and hatchlings are able to capture prey in the same manner as the adults, although a large quantity of yolk is still stored in the internal yolk sac (Boucaud-Camou *et al.*, 1985; Vecchione, 1987). On the other hand, although little is known about paralarval feeding of the Oegopsida, *T. pacificus* is estimated to start feeding at about stage 32 (five days after hatching) when the digestive organs are well differentiated. At this stage, the stomach exhibits peristaltic movement, and secretion of enzymes is observed in the salivary glands. *T. pacificus* could live on yolk absorption for a few days after hatching, and could immediately shift to exogenous feeding as soon as the yolk is absorbed. O'Dor *et al.* (1985) have suggested *Illex* rhynchoteuthion paralarvae have the capacity for suspension feeding. It is likely that small premature paralarvae of oceanic squids (Table 1) have a special paralarval feeding habit, given such

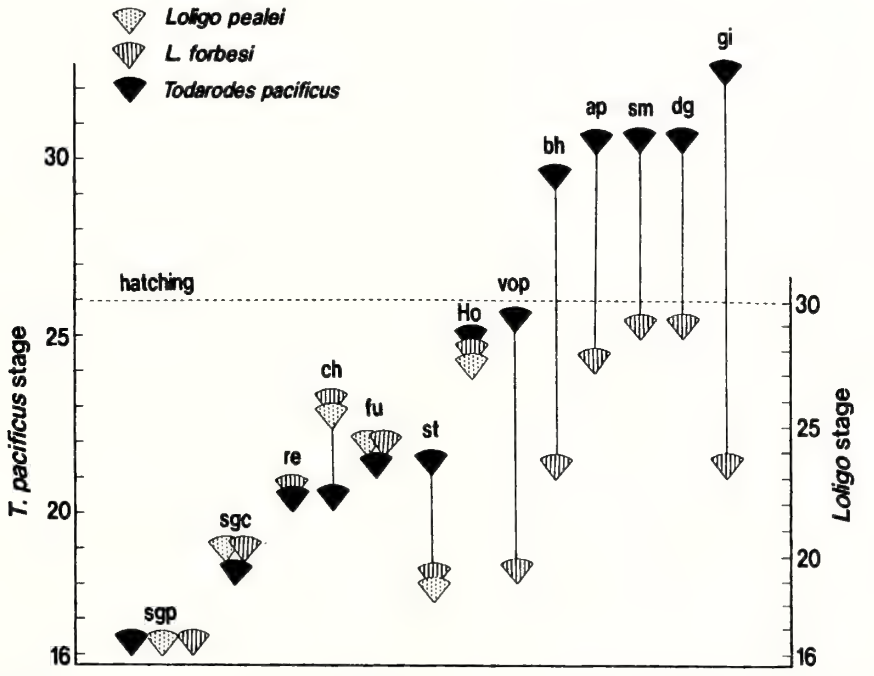

Fig. 11. The sequence of appearance of major features in *Loligo pealeii* (*fide* Arnold, 1965), *L. forbesi* (*fide* Segawa *et al.*, 1988), and *Todarodes pacificus* in the present study. For comparison, two stages are used for major criteria: stage 16 at commencement of organogenesis and the stage of hatching. (ap, anal papilla; bh, branchial heart; ch, chromatophore; dg, digestive gland; fu, funnel; gi, gill; Ho, Hoyle's organ; re, retina; sgc, closure of shell gland; sgp, primordium of shell gland; sm, stomach; st, statocyst; vop, primordium of ventral organ as three swellings, such as gills, branchial hearts, systemic heart, stomach, caecum, and anal knoll).

Table 1. Summary of egg size, number of eggs per spawning, and embryonic development time of 13 species of cephalopods. (–, no data).

Taxa	Egg size (mm)	Number of eggs per spawning	Days required for hatching (plus water, temperature, °C)	Hatchling size (mm ML)	Reference*
Sepia lycidas Gray, 1849	29.0 x 12.5	–	–	–	1
S. latimanus Quoy and Gaimard, 1832	28.5 x 22.3	30-40	about 40	13-14	1, 2
S. esculenta Hoyle, 1885	17.5 x 13.0	500	40 (18°C)	–	1
Sepiella japonica Sasaki, 1929	9.5 x 7.0	100s	35 (21°C)	3.4-4.3	3
Euprymna scolopes Berry, 1913	–	200	18 (24°C)	1.6	4
Idiosepius paradoxus Ortmann, 1881	1.5 x 1.3	–	16 (20°C)	1.16-1.22	5
Sepioteuthis lessoniana Lesson, 1830	5.8 x 5.6	500-1,000	25 (25°C)	5-7	6
Loligo pealeii LeSueur, 1821	1.6 x 1.0	–	–	1.6	7
L. bleekeri Keferstein, 1866	2.7 x 2.6	1,200-2,000	64-67 (18°C)	3.0-3.3	8, 9
L. forbesi Steenstrup, 1856	3.3 x 3.0	–	68-75 (25°C)	4.3-4.9	10
Watasenia scintillans (Berry, 1911)	1.5 x 1.2	2,000	5 (18°C)	1.4	11
Illex illecebrosus (LeSueur, 1821)	0.9 x 0.7	100,000	9 (21°C)	1.1	12
Todarodes pacificus Steenstrup, 1880	0.8 x 0.7	200,000†	4 (20°C)	0.95	13

*1, Okutani, 1978; 2, Corner and Moore, 1980; 3, Yamamoto, 1982; 4, Arnold *et al.*, 1972; 5, Natsukari,1970; 6, Segawa, 1987; 7, Boletzky and Hanlon, 1983; 8, Baeg *et al.*, 1992; 9, Natsukari and Tashiro, 1991; 10, Segawa *et al.*, 1988; 11, S. Hayashi, 1995; 12, O'Dor *et al.*, 1982; 13, Present study, and Bower and Sakurai,1996 (†).

a rapid shift from endogenous to exogenous feeding.

In *Todarodes pacificus,* development of the gills is remarkably delayed in spite of their later importance as vital organs. In embryos with a large external yolk sac, *viz.* the Myopsida and Sepioidea, the gills are fully developed long before hatching, and oxygen-rich blood fills the sinus of the external yolk sac before final development of the gills (Boletzky, 1987). In such embryos, the external yolk sac already functions as a pump before the development of the systemic heart and branchial hearts in later organogenesis (Boletzky, 1989). In contrast, *T. pacificus* embryos have no blood sinus in the external yolk sac and the final development of the gills is very late. The embryos and hatchlings of *T. pacificus* should achieve dermal respiration through the surface tissues by the final development of gills as was assumed by Arnold and O'Dor (1990).

In conclusion, the paralarval stages of *Todarodes pacificus* are characterized by special developmental patterns in digestive and respiratory systems as well as by some external morphological characters, such as formation of a so-called proboscis. A developmental process in which hatching occurs before the completion of some important organs could characterize the Oegopsida which produces small eggs, have a short embryonic developmental time, and small hatchlings as compared with the Myopsida and Sepioidea (Table 1).

In the Cephalopoda, egg size and fecundity vary among species (Table 1). In general the Sepioidea and Myopsida tend to produce fewer but proportionally larger eggs and developmental time is longer, as compared with the Oegopsida. The Sepioidea and Myopsida embryos have a large external yolk sac at later embryonic stages, and the yolk therein is gradually transferred into the internal yolk sac during stages 25-30 of Arnold (1965). In this process, the shape of the internal yolk sac becomes rather complicated. On the other hand, although embryos of *Todarodes pacificus* do not have such a distinct external yolk sac, they do have a dome-shaped external yolk sac, and a small-scale transfer of yolk is observed during stages 24-28. Observations of morphological changes in the yolk sac from nine species of the families Sepiidae, Sepiolidae, Loliginidae, Enoploteuthidae, Ommastrephidae, and Thysanoteuthidae (Watanabe, unpub.), show three families belonging to the Oegopsida obviously have no distinct external yolk sac except for a dome-shaped external yolk sac. And from this observation, it is clear that each taxon has its own pattern of morphological change in the internal yolk sac. The patterns of the morphological changes of the Oegopsida are simpler than those of the Sepioidea and Myopsida. The only morphological change of the internal yolk sac in *T. pacificus* is the formation a gutter by the esophagus, in contrast to several gutters formed by blood vessels as well as the alimentary canal in the Sepioidea and Myopsida. This morphological change of the internal yolk sac could play a dominant role in the development of circulatory organs, because the sinus surrounding the internal yolk sac differentiates a network of vessels (Boletzky, 1989). The morphological change of the yolk sac is reflected by the location of organs around the yolk sac in the embryonic phase, including the vessels. In approaching a phylogeny of cephalopods from an embryological point of view, comparative study of morphological change of the yolk sac could be important.

From such a comparative point of view, the Oegopsida including *Todarodes pacificus* which produce a large number of small eggs are specialized among the Recent cephalopods by the presence of a reduced external yolk sac, and delayed differentiation of some organs, such as the respiratory and digestive organs. These characters could reflect a reproductive strategy for paralarval dispersion in the open ocean.

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LITERATURE CITED

Arnold, J. M. 1965. Normal embryonic stages of the squid, *Loligo pealii* Lesueur. *Biological Bulletin* 128(1):24-32.

Arnold, J. M. 1990. Embryonic development of the squid. *In: Squid as Experimental Animals*, D. L. Gilbert, W. J. Adelman, Jr., and J. M. Arnold, eds. pp. 77-90. Plenum Press, New York.

Arnold, J. M. and R. O'Dor. 1990. In vitro fertilization and embryonic development of oceanic squid. *Journal of Cephalopod Biology* 1(2):21-36.

Arnold, J. M., C. T. Singley, and L. D. Williams-Arnold. 1972. Embryonic development and post-hatching survival of the sepiolid squid *Euprymna scolopes* under laboratory conditions. *The Veliger* 14(4):361-364.

Baeg, G. H., Y. Sakurai, and K. Shimazaki. 1992. Embryonic stages of *Loligo bleekeri* Keferstein. *The Veliger* 35(3):234-241.

Boletzky, S. v. 1987. Ontogenetic and phylogenetic aspects of the cephalopod circulatory system. *Experientia* 43:478-483.

Boletzky, S. v. 1988. Cephalopod development and evolutionary concepts. *In: The Mollusca, Vol. 12. Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 185-202. Academic Press, London.

Boletzky, S. v. 1989. Recent studies on spawning, embryonic development, and hatching in Cephalopoda. *In: Advances in Marine Biology, Vol. 25,* J. H. S. Blaxter and A. J. Southward, eds. pp. 85-115. Academic Press, London.

Boletzky, S. v. and R. T. Hanlon. 1983. A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. *Memoirs of the National Museum of Victoria* 44:147-187.

Boletzky, S. v., L. Rowe, and L. Aroles. 1973. Spawning and development of the eggs, in the laboratory, of *Illex coindetii* (Mollusca: Cephalopoda). *The Veliger* 15:257-258.

Boucaud-Camou, E., M. Yim, and A. Tresgot. 1985. Feeding and digestion of young *Sepia officinalis* L. (Mollusca: Cephalopoda) during post-hatching development. *Vie et Milieu* 35(3/4):263-266.

Bower, J. R. and Sakurai, Y. 1996. Laboratory observations on *Todarodes pacificus* (Cephalopoda: Ommastrephidae) egg masses. *American Malacological Bulletin* 13(1/2):65-71.

Corner, B. D. and H. T. Moore. 1980. Field observations on the reproductive behavior of *Sepia latimanus. Micronesia* 16(2):235-260.

Hamabe, M. 1961. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. Early embryonic development and morphological features of the larvae immediately after hatching.] *Zoological Magazine* 70(12):408-420. [In Japanese with English title and abstract].

Hamabe, M. 1962. [Embryological studies on the common squid, *Ommastrephes sloani pacificus* Steenstrup, in the southwestern waters of the Sea of Japan.] *Bulletin of Japan Sea Regional Fisheries Research Laboratory* 10:1-45. [In Japanese with English title and abstract].

Hayashi, H. 1960. [Development of the squid, *Ommastrephes sloani pacificus.*] *Bulletin of Faculty of Fisheries, Nagasaki University* 9:43-47. [In Japanese with English title and abstract].

Hayashi, S. 1995. [Fishery biological studies of the firefly squid, *Watasenia scintillans*, in Toyama Bay.] *Bulletin of Toyama Prefectural Fisheries Research Institute* 7:1-128. [In Japanese with English title and abstract].

Ikeda, Y., Y. Sakurai, and K. Shimazaki. 1993. Fertilizing capacity of squid *(Todarodes pacificus)* spermatozoa collected from various sperm storage sites, with special reference to the role of gelatinous substance from oviducal gland in fertilization and embryonic development. *Invertebrate Reproduction and Development* 23(1):39-44.

Naef, A. 1928. Die Cephalopoden. *Fauna Flora Golf Neapel* 35(2):1-357.

Natsukari, Y. 1970. [Egg-laying behavior, embryonic development and hatched larva of the pygmy cuttlefish, *Idiosepius pygmaeus paradoxus* Ortmann.] *Bulletin of the Faculty of Fisheries, Nagasaki University* 30:15-29. [In Japanese with English title and abstract].

Natsukari, Y. and M. Tashiro. 1991. Neritic squid resources and cuttlefish resources in Japan. *Marine Behaviour and Physiology* 18:149-226.

O'Dor, R. K., N. Balch, E. A. Foy, R. W. M. Hirtle, D. A. Johnston, and T. Amaratunga. 1982. Embryonic development of the squid, *Illex illecebrosus*, and effect of temperature on development rates. *Journal of North-west Atlantic Fishery Science* 3:41-45.

O'Dor, R. K., P. Helm, and N. Balch. 1985. Can rhynchoteuthion suspension feed? *Vie et Milieu* 35(3/4):267-271.

Okutani, T. 1978. [Studies on early life history of decapodan Mollusca - VII. Eggs and newly hatched larvae of *Sepia latimanus* Quoy and Gaimard.] *Venus* 37(4):245-248. [In Japanese with English title and abstract].

Okutani, T. 1995. *Cuttlefish and Squids of the World in Color.* National Cooperative Association of Squid Processors, Tokyo. 185 pp.

Okutani, T., M. Tagawa, and H. Horikawa. 1987. *Cephalopods from Continental Shelf and Slope around Japan.* Japan Fisheries Resource Conservation Association, Tokyo. 194 pp.

Sakurai, Y, J. R. Bower, Y. Nakamura, S. Yamamoto, and K. Watanabe. 1996. Effect of temperature on development and survival of *Todarodes pacificus* embryos and paralarvae. *American Malacological Bulletin* 13(1/2):89-95.

Sakurai, Y. and Y. Ikeda. 1992. [Laboratory rearing methods of *Todarodes pacificus* for the ecological study of life cycle.] Proceedings of the Squid Stock and Fisheries Oceanic Conditions. *National Research Institute of Far Seas Fisheries* (1993):51-69. [In Japanese with English title].

Sakurai, Y., R. E. Young, J. Hirota, K. Mangold, M. Vecchione, M. R.

Clarke, and J. Bower. 1995. Artificial fertilization and development through hatching in the oceanic squids *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* (Cephalopoda: Ommastrephidae). *The Veliger* 38(3):185-191.

Segawa, S. 1987. Life history of the oval squid, *Sepioteuthis lessoniana* in Kominato and adjacent waters central Honshu, Japan. *Journal of the Tokyo University of Fisheries* 74(2):67-105.

Segawa, S., W. T. Yang, H. J. Marthy, and R. T. Hanlon. 1988. Illustrated embryonic stages of the eastern Atlantic squid *Loligo forbesi*. *The Veliger* 30(3):230-243.

Soeda, J. 1952. [On the artificial insemination and the early cleavage of the ovum in oegopsid Cephalopoda, *Ommastrephes sloani pacificus*, Surume-ika.] *Bulletin of the Hokkaido Regional Fisheries Laboratory* 5:1-15. [In Japanese with English title and abstract].

Soeda, J. 1954. [A study on the fertilization of the egg of the squid, *Ommastrephes sloani pacificus* (Steenstrup), Surume-ika.] *Bulletin of the Hokkaido Regional Fisheries Laboratory* 11:1-6. [In Japanese with English title and abstract].

Yamamoto, M. 1982. [Normal stages in the development of the cuttlefish, *Sepiella japonica* Sasaki.] *Zoological Magazine* 91:146-157. [In Japanese with English title and abstract].

Vecchione, M. 1987. Juvenile ecology. *In: Cephalopod Life Cycles, Vol. 2*, P. R. Boyle, ed. pp. 61-84. Academic Press, London.

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Effect of temperature on development and survival of *Todarodes pacificus* embryos and paralarvae

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Abstract: Embryonic development and survival of paralarvae of the Japanese common squid, *Todarodes pacificus* (Steenstrup, 1880), were examined at 16 temperatures (3-29°C) to determine the optimum temperature range for development and survival. Normal embryonic development occurred at temperatures between 14.0° and 26.0°C, with highest embryonic survival rates occurring between 14.7° and 22.2°C. The relationship between temperature (T) and the number of days from artificial fertilization to hatching (D) is expressed by a polynomial function: $\ln(D) = 4.73 - 0.227(T) + 0.00304(T^2)$. A modified formula, based on observations of two egg masses spawned in captivity, was used to estimate the development rate of eggs within naturally spawned egg masses. It is suggested that *T. pacificus* spawns in waters warmer than 12.1°C, and egg masses maintain their structure for 4.0 - 9.5 days before disintegrating at temperatures between 14.7° and 22.2°C. Paralarvae survived up to 13 days after hatching, with the highest survival rates occurring at 15°C.

Key words: squid, paralarva, temperature, artificial fertilization

The Japanese common squid, *Todarodes pacificus* (Steenstrup, 1880), performs seasonal migrations in waters near Japan, with spawning occurring at the southern end of its distribution (Okutani, 1983; Murata, 1989, 1990). There have been no observations of *T. pacificus* egg masses in the sea, but laboratory observations indicate they produce nearly neutrally buoyant, spherical egg masses, up to 80 cm in diameter, each with approximately 200,000 eggs (Bower and Sakurai, 1996). The main spawning activity occurs during autumn and winter (Araya, 1976; Okutani, 1983). During this period, the main spawning groups gradually shift southward from the southwestern part of the Sea of Japan to the northern part of the East China Sea (Murata, 1989, 1990). This shift in the location of the spawning grounds could be due to seasonal changes of sea temperature in the region.

The embryonic development of cephalopods is highly temperature dependent (Boletzky, 1987). Laboratory experiments on the ommastrephid squids *Illex illecebrosus* (LeSueur, 1821) (O'Dor *et al.*, 1982) and *Ommastrephes bartrami* (LeSueur, 1821) (Sakurai *et al.*, 1995) indicate that in both species, the rate of embryonic development increases with temperature. O'Dor *et al.* (1982) examined the effect of temperature on the embryonic development rates of artificially and naturally spawned eggs of *I. illecebrosus* and found that embryonic development failed below 12.5°C. The authors suggested that the spatial and temporal distribution of spawning is restricted by this temperature requirement. A temperature minimum above 10°C has also been reported for *Illex coindetii* (Verany, 1839) (Boletzky *et al.*, 1973).

Hayashi (1960) was the first to rear embryos of *Todarodes pacificus* through hatching. At water temperatures from 9.8-13.2°C, the development of many fertilized embryos stopped at early developmental stages. Later studies revealed that successful hatching of naturally spawned eggs can occur at 14-21°C (Hamabe, 1961a, b, c, 1962). The difficulty of both finding egg masses in nature and maintaining hatchling paralarvae has severely limited any further study of embryonic development and the early life stages.

With the recent development of a method for rearing ommastrephid paralarvae through artificial fertilization (Sakurai *et al.*, 1995), more extensive study of the development and survival of eggs and paralarvae is now possible. This paper presents results of experiments examining the effect of temperature on the development and survival of *Todarodes pacificus* embryos and paralarvae reared through artificial fertilization. Inferences are then made about the location of spawning and egg masses in nature.

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MATERIALS AND METHODS

Adult squid were collected from set trap nets and by hand jigging from the inshore waters of southern Hokkaido, Japan, during 1992 to 1994. The squid were maintained in a raceway tank (5.5 m in length, 2.5 m in width, 1.2 m in depth, and 13,000 l in capacity) at the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University. The maintenance procedure followed that described by Sakurai *et al.* (1993). The on-off cycle of the timer-controlled halogen white lamps coincided with the diurnal photoperiod (10-13 hrs light: 11-14 hrs dark). Water temperatures ranged from 15.8-18.5°C, and salinities from 32-34 ppt.

Four live females with mature eggs ovulated in the oviducts were transported from the Usujiri Laboratory to the main campus of the Faculty of Fisheries, Hokkaido University, for artificial fertilization experiments in November 1992, September 1993, and November 1994 (Table 1). The technique used to fertilize eggs removed from mature females was described by Sakurai *et al.* (1995). The procedure, which required approximately 20 min per individual female, was conducted at room temperatures (15-20°C). Petri dishes containing 100-300 fertilized eggs were maintained in incubators at 3.5°, 7.1°, 10.0°, 12.1°, 14.0°, 14.7°, 15.0°, 16.9°, 18.9°, 19.9°, 21.3°, 22.2°, 23.2°, 24.5°, 26.0°, and 29.0°C. The fertilization rate at each temperature was determined based on the percentage of normally developing eggs calculated from several dishes one day after fertilization. Dishes were selected randomly and examined under a dissecting microscope.

During the 1992 experiments, after determining the fertilization rate, the development of 4,971 eggs (461-912 eggs per temperature unit) was examined at eight temperature units (3.5°, 7.1°, 10.0°, 12.1°, 14.0°, 24.5°, 26.0°, and 29.0°C) by placing 3-5 eggs in separate cell-tray containers (3 ml filtered seawater/cell). The filtered seawater was changed two times a day, at which time the developmental stages were observed and the dead eggs and paralarvae were counted and removed. After hatching, observation of the paralarvae continued twice daily until all paralarvae were dead. No attempts were made to feed the paralarvae. Because change of seawater caused the dilution of oviducal gland jelly and physical shock to the eggs, and oxygen did not appear to be a limiting factor, the procedure was slightly modified during the 1993 and 1994 experiments. At each temperature unit, the development and survival of eggs were examined by placing 10-20 eggs in 100-mm-diameter petri dishes. Seawater was changed once a day. In 1993, the development of 2,410 eggs (769-829 eggs per temperature unit) was examined at three temperature units (14.7°, 19.9°, and 23.2°C). In 1994, the development of 4,880 eggs (869-1,123 eggs per temperature unit) was examined at five temperature units (15.0°, 16.9°, 18.9°, 21.3°, and 22.2°C).

Embryonic development rates were compared at different temperatures. Embryos were classified within the scheme of developmental stages given by Watanabe *et al.* (1996). The period of embryonic development was defined

Table 1. Summary of *Todarodes pacificus* artificial fertilization experiments and embryonic development at different temperatures. Female DML, dorsal mantle length of adult female used for artificial fertilization.

Mean temp (°C)	Fertilization date	Female DML (mm)	No. eggs	No. fertilized eggs	No. embryos surviving to 50% hatching date
3.5	09 November 1992	240	506	0	–
7.1	09 November 1992	240	518	500	0
10.0	19 November 1992	252	675	649	0
12.1	19 November 1992	252	912	872	100
14.0	19 November 1992	252	896	846	389
14.7	18 September 1993	278	769	750	592
15.0	08 November 1994	290	1,055	981	930
16.9	08 November 1994	290	947	856	733
18.9	08 November 1994	290	869	781	739
19.9	18 September 1993	278	829	783	642
21.3	08 November 1994	290	1,123	1,101	979
22.2	08 November 1994	290	886	844	618
23.2	18 September 1993	278	812	776	372
24.5	19 November 1992	252	498	483	116
26.0	19 November 1992	252	505	494	99
29.0	19 November 1992	252	461	0	–
Totals			12,261	10,716	6,309

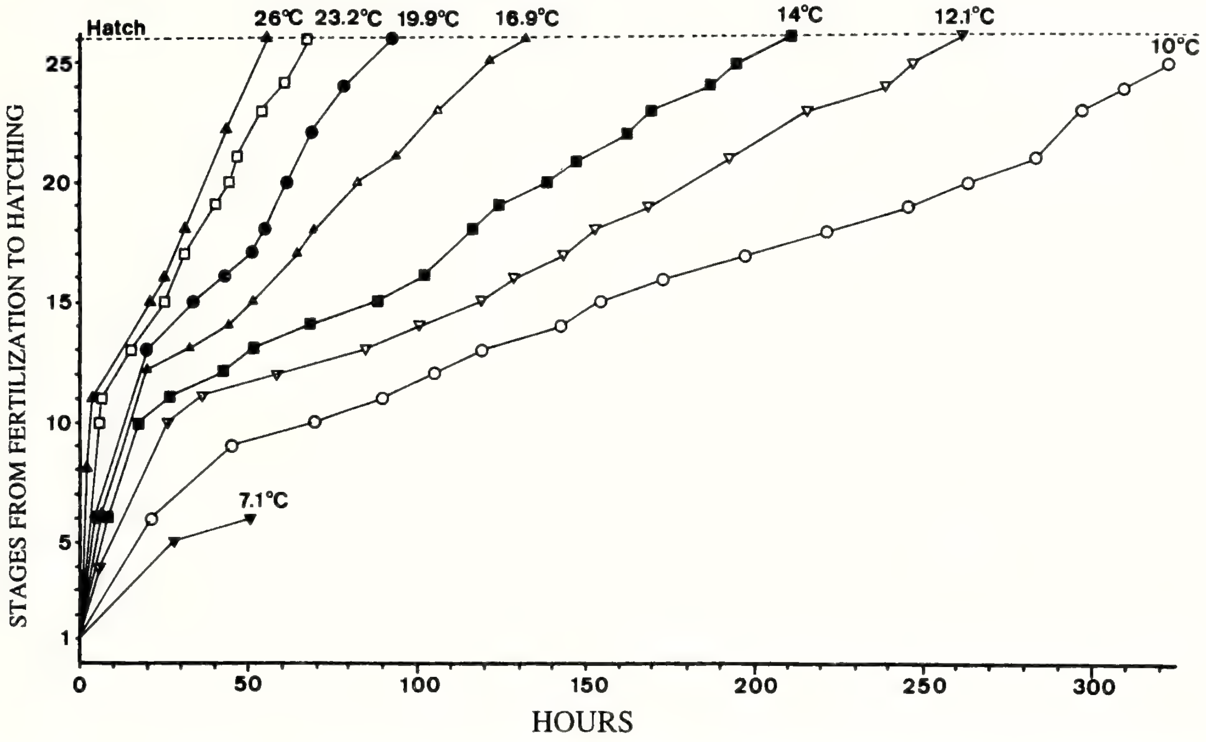

Fig. 1. Course of embryonic development at eight incubation temperatures in *Todarodes pacificus*. Stage criteria applied are from Watanabe *et al.* (1996).

Table 2. Fertilization rates and embryonic survival rates at the 50% hatching date for each incubation temperature examined. ----, no hatching.

Temperature (°C)	Fertilization rate (%)	Survival rate at 50% hatching (%)	Remarks
3.5	0	----	no blastoderm formation
7.1	96.5	----	no development after Stage 6
10.0	96.1	----	no development after Stage 25
12.1	95.6	11.5	no arm development; some paralarvae survive 6 days
14.0	94.4	46.0	development normal
14.7	97.5	78.9	development normal
15.0	93.0	94.8	development normal
16.9	90.4	85.6	development normal
18.9	89.9	94.6	development normal
19.9	94.5	82.0	development normal
21.3	98.0	88.9	development normal
22.2	95.3	73.2	development normal
23.2	95.6	47.9	some paralarvae hatch with inverted mantles
24.5	97.0	24.0	many paralarvae hatch with inverted mantles
26.0	97.8	20.0	development abnormal at late stages
29.0	0	----	no blastoderm formation

as the time from fertilization until point of "50% hatching." "50% hatching" was defined as the point when the number of hatched embryos equals the number of live unhatched embryos. The developmental period of artificially fertilized eggs was also compared with the developmental period of eggs within egg masses spawned in captivity (Bower and Sakurai, 1996).

RESULTS

EMBRYOS

Fertilization rates in the artificial fertilization experiments ranged from 89.9-98%, except at 3.5° and 29°C, where fertilization did not occur (Table 2). Although fertilization did occur at 7.1° and 10.0°C, no embryos survived through hatching at temperatures below 12.1°C. The maximum temperature for fertilization and development was 26.0°C.

The course of embryonic development of *Todarodes pacificus* at eight incubation temperatures is shown in Fig. 1. Development was highly temperature dependent. At 7.1° and 10°C, development ceased at Stage 6 and Stage 25, respectively. At 12.1°C, arms did not develop, but several paralarvae survived up to six days after hatching. At 23.2° and 24.5°C, the mantles of most developing larvae were completely inverted. These paralarvae had their viscera exposed, were unable to swim, and survived less than one day after hatching.

Table 3. Estimated number of days to hatching from a *Todarodes pacificus* natural egg mass, based on the number of days to 50% hatching of artificially fertilized eggs at eleven different incubation temperatures.

Temperature (°C)	Number of days from fertilization to 50% hatching artificial fertilization	calculated from regression[a]	estimated for egg mass[b]
12.1	11.5	11.3	13.9
14.0	8.4	8.6	10.5
15.0	7.7	7.5	9.1
16.9	5.5	5.8	7.1
18.9	4.5	4.6	5.6
19.9	4.0	4.1	5.0
21.3	3.7	3.3	4.0
23.2	3.2	3.0	3.7
24.5	2.5	2.7	3.3
26.0	2.3	2.4	3.0

[a] $\ln(\text{day}) = 4.73 - 0.227(x) + 0.00304(x)^2$ ($r^2 = 0.993$).
[b] $\ln(\text{day}) = 4.93 - 0.227(x) + 0.00304(x)^2$.

The number of days from fertilization to 50% hatching of artificially fertilized eggs (D) was plotted against incubation temperature (T) in Fig. 2. The relationship between temperature and embryonic development can be expressed as a polynomial function: $\ln(D) = 4.73 - 0.227(T) + 0.00304(T^2)$. In 1994, observation of hatching from two *Todarodes pacificus* egg masses spawned in captivity indicated the peak of hatching occurred about 5.5 days after fertilization at 19°C (Bower and Sakurai, 1996), or approximately one day later than that predicted for artificially fertilized eggs. The formula for hatching from an egg mass was modified based on this single observation: $\ln(D) = 4.93 - 0.227(T) + 0.00304(T^2)$.

From these two equations, the number of days from fertilization to 50% hatching at temperatures between 12.1° and 26.0°C were estimated for both artificially fertilized eggs and eggs within an egg mass (Table 3). At 14.0°C, the lowest temperature of normal paralarval hatching, development within an egg mass was estimated to require 10.5 days. At 26°C, the highest temperature of paralarval hatching, hatching within an egg mass was estimated to occur 3.0 days after fertilization. The highest survival rates at 50% hatching (> 70%) occurred between 14.7° and 22.2°C. Hatching from an egg mass over this temperature range was estimated to occur between 4.0 and 9.5 days.

The sequential changes in egg and paralarval mortality at temperatures from 12.1° to 18.9°C are shown in Fig. 3. Through this temperature range, mortality rates before hatching were below 20% from 14.7-18.9°C, but over 60% at 12.1° and 14°C. The sequential changes in egg and paralarval mortality at temperatures from 19.9-24.5°C are shown in Fig. 4. Mortality rates before hatching were higher at 23.2° and 24.5°C than at 19.9° and 22.2°C.

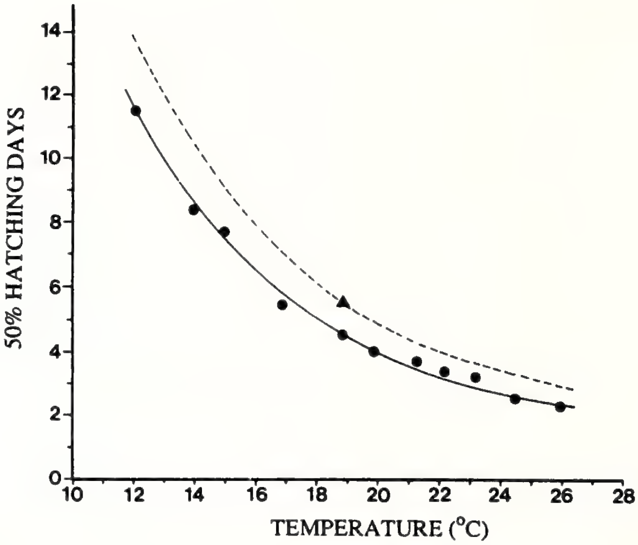

Fig. 2. Relationship between number of days to 50% hatching and temperature for artificially fertilized *Todarodes pacificus* eggs. Solid line represents the regression of a polynomial function for artificially fertilized embryos: $\ln(y) = 4.73 - 0.227(x) + 0.00304(x^2)$ ($r^2 = 0.993$). Broken line represents the proposed regression for naturally spawned eggs, based on single observation of 5.5 days at 19°C (triangle): $\ln(y) = 4.93 - 0.227(x) + 0.00304(x^2)$.

PARALARVAE

Fig. 5 shows the changes in survival rates at 1-3 day intervals of *Todarodes pacificus* paralarvae from the 50% hatching date until 11 days post-hatching at temperatures from 12.1-26.0°C. The peak of paralarval survival occurred between 14.7° and 22.2°C. Survival rates of embryos until hatching were over 70% across this temperature range. With the exception of 19.9°C, survival rates of paralarvae between 14.7° and 22.2°C were greater than 50% at three days after the 50% hatching date. It is not known why survival rates at 19.9°C were anomalously lower than the overall pattern would predict. The lower temperature boundary for paralarval survival was evident at 14-15°C.

DISCUSSION

The present study demonstrates that normal embryonic development through hatching in *Todarodes pacificus* occurs over a temperature range of 14.0-26.0°C, with highest survival rates (above 70%) occurring between 14.7° and 22.2°C. Hamabe (1961c, 1962) successfully reared *T. pacificus* paralarvae over a similar temperature range (14-21°C), and noted that abnormal development was common at 19-25°C, and most embryos died before hatching at temperatures over 25°C. In the present study, the inversion of mantles of developing embryos at temperatures

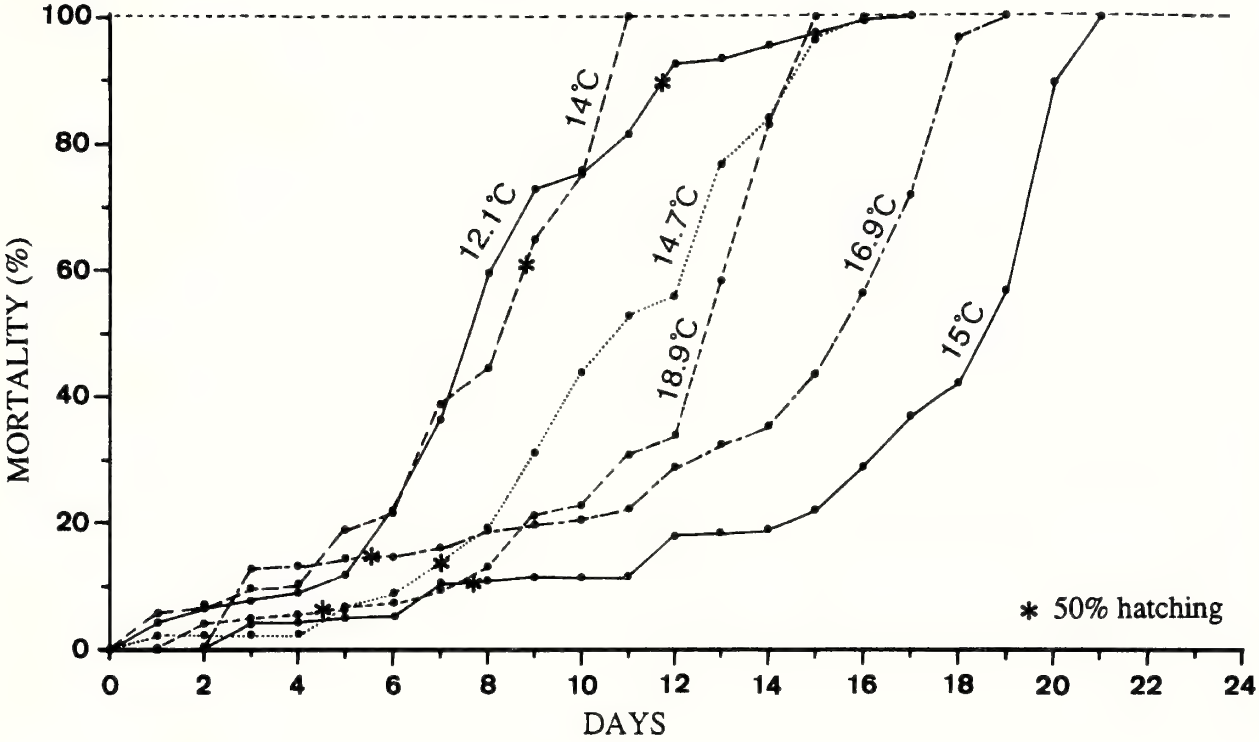

Fig. 3. *Todarodes pacificus* egg and paralarval mortality rates at temperatures between 12.1° and 18.9°C. *, 50% hatching (see text for explanation).

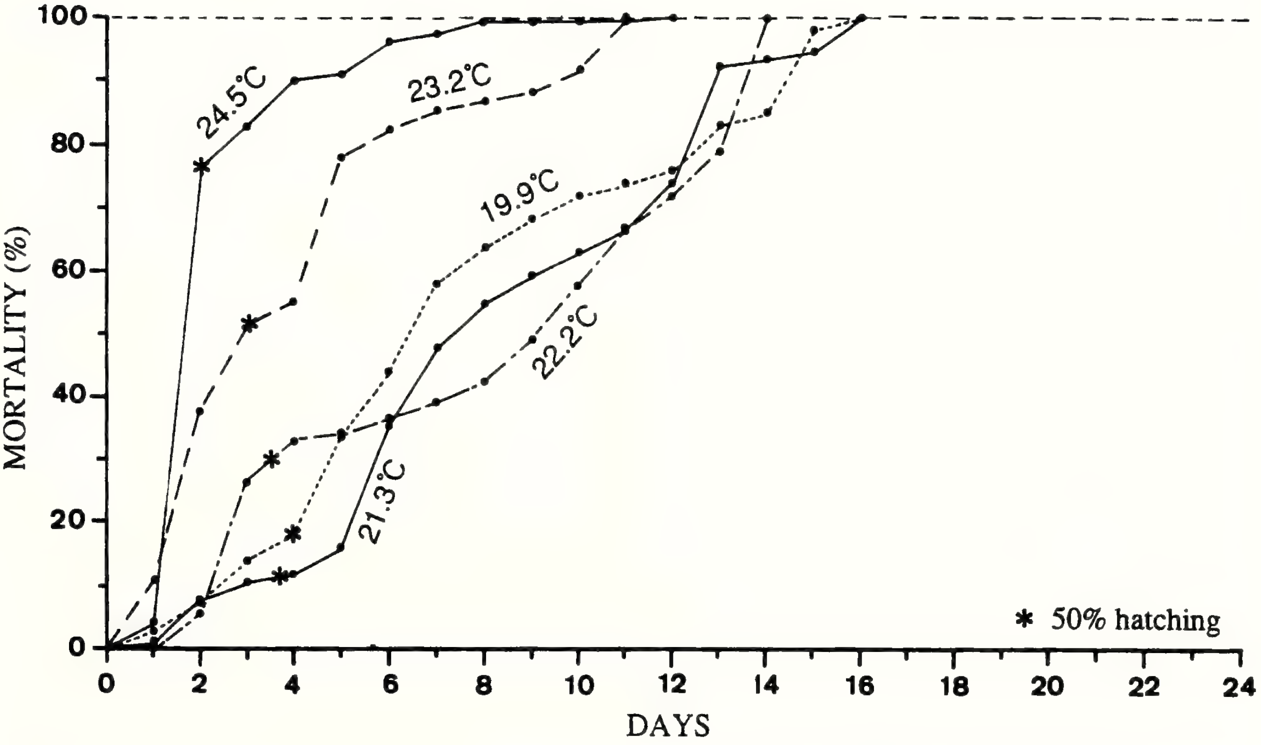

Fig. 4. *Todarodes pacificus* egg and paralarval mortality rates at temperatures between 19.9° and 24.5°C. *, 50% hatching (see text for explanation).

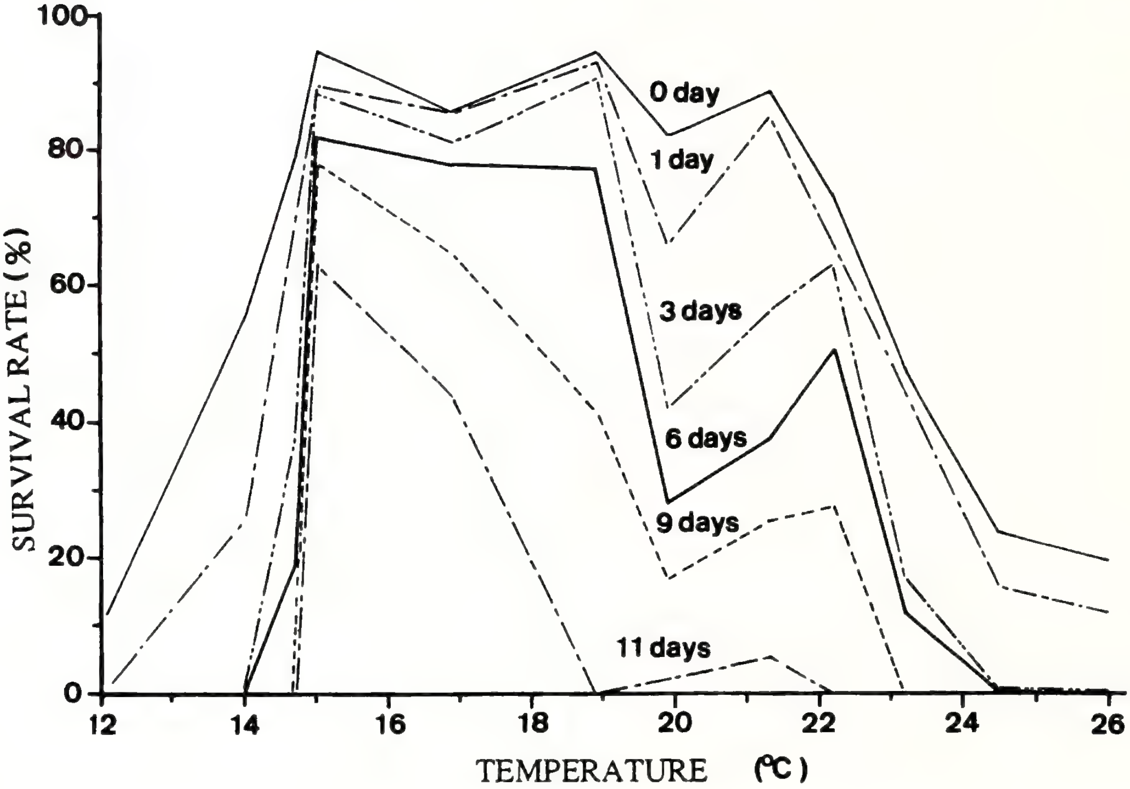

Fig. 5. Survival rates at 1-3 day intervals of *Todarodes pacificus* paralarvae from the 50% hatching date to 11 days post-hatching at temperatures between 12° and 26°C.

above 22.2°C could have been due to hyperembryogenesis at warmer temperatures. At temperatures below 12.1°C, arm development did not occur during the early stages. The lower temperature range (9.8-13.2°C) used by Hayashi (1960) probably caused the low embryonic survival rates.

Comparison of the development rates of artificially fertilized eggs and eggs within spawned egg masses indicated that hatching occurs approximately one day later within egg masses. O'Dor *et al.* (1982) also reported delayed hatching from *Illex illecebrosus* egg masses. Watanabe *et al.* (1996) confirmed that artificially fertilized paralarvae hatched earlier (Stages 25-26) than egg-mass paralarvae (Stage 28). Choe (1966) reported that a mechanical stimulus can elicit premature hatching in developing cephalopod embryos. The protection provided by the enveloping jellies from the oviducal and nidamental glands that surround the egg-mass embryos is presumably responsible for the delay in hatching.

Bower and Sakurai (1996) reported *Todarodes pacificus* paralarvae that hatched from egg masses and swam freely within a raceway tank at 19°C died approximately 6-7 days after hatching. Balch *et al.* (1985) also reported that *Illex illecebrosus* paralarvae hatched from egg masses died within a week. In the present study, however, paralarvae survived up to 13 days after hatching, with the highest survival rates occurring at 15°C. The longer survival times of artificially fertilized paralarvae at similar temperatures from the present experiment were presumably due to the lower metabolic demands on paralarvae in petri dishes than free-swimming paralarvae, or to larger yolk volume at hatching.

With the onset of autumn and the decrease of water temperatures on the main feeding grounds near northern Japan, the main population (autumn and winter spawning groups) of *Todarodes pacificus* begins a migration to the spawning grounds near southern Japan (Okutani, 1983; Murata, 1989, 1990). During winter, water temperatures on the feeding grounds drop below 5°C (K. Shimazaki, pers. comm.). A factor responsible for the southern migration appears to be the need for waters between 14.0° and 26.0°C for spawning.

The 14.0-26.0°C temperature range of normal development will delimit the timing and location of spawning of this squid, and the location of egg masses in nature. Fertilization rates were high over a wide range of temperatures (7.1-26°C). Thus spawning could occur over this wide temperature range. However highest embryonic survival rates (above 70%) occurred between 14.7° and 22.2°C.

Bower and Sakurai (1996) reported egg masses disintegrated soon after hatching. Thus, based on the 14.7-22.2°C temperature range, egg masses in nature are estimated to maintain their shape for only 4.0 to 9.5 days.

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LITERATURE CITED

Araya, H. 1976. [Migration and fishing ground of winter sub-population of the squid *Todarodes pacificus* Steenstrup in the northern waters of Japan.] *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 41:119-129. (In Japanese with English abstract).

Balch, N., R. K. O'Dor, and P. Helm. 1985. Laboratory rearing of rhynchoteuthions of the ommastrephid squid *Illex illecebrosus* (Mollusca: Cephalopoda). *Vie et Milieu* 35(3/4):243-246.

Boletzky, S. v. 1987. Embryonic phase. *In*: *Cephalopod Life Cycles, Vol. 2,* P. R. Boyle, ed. pp. 5-31. Academic Press, London.

Boletzky, S. v., L. Rowe, and L. Aroles. 1973. Spawning and development of the eggs, in the laboratory, of *Illex coindetii* (Mollusca: Cephalopoda). *The Veliger* 15(3):257-258.

Bower, J. R. and Y. Sakurai. 1996. Laboratory observations on *Todarodes pacificus* (Cephalopoda: Ommastrephidae) egg masses. *American Malacological Bulletin* 13(1/2):65-71.

Choe, S. 1966. On the eggs, rearing, habits of the fry, and growth of some Cephalopoda. *Bulletin of Marine Science* 16:330-348.

Hamabe, M. 1961a. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. I. Copulation.] *Zoological Magazine* 70(12):378-384. (In Japanese with English abstract).

Hamabe, M. 1961b. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. II. Spawning behavior.] *Zoological Magazine* 70(12):385-394. (In Japanese with English abstract).

Hamabe, M. 1961c. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. III. Early embryonic development and morphological features of the larvae immediately after hatching.] *Zoological Magazine* 70(12):408-420. (In Japanese with English abstract).

Hamabe, M. 1962. [Embryological studies on the common squid, *Ommastrephes sloani pacificus* Steenstrup, in the southwestern waters of the Sea of Japan.] *Bulletin of the Japanese Regional Fisheries Research Laboratory* 10:1-45. (In Japanese with English abstract).

Hayashi, S. 1960. [Development of the squid, *Ommastrephes sloani pacificus* (Steenstrup).] *Bulletin of Faculty of Fisheries, Nagasaki University* 9:43-51. (In Japanese with English abstract).

Murata, M. 1989. Population assessment, management and fishery forecasting for the Japanese common squid, *Todarodes pacificus*. *In: Marine Invertebrate Fisheries: Their Assessment and Management,* J. R. Caddy, ed. pp. 613-636. John Wiley and Sons, New York.

Murata, M. 1990. Oceanic resources of squids. *Marine Behavior and Physiology* 18:19-71.

O'Dor, R. K., N. Balch, E. A. Foy, R. W. M. Hirtle, D. A. Johnstone, and T. Amaratunga. 1982. Embryonic development of the squid, *Illex illecebrosus*, and the effect of temperature on development rates. *Journal of Northwest Atlantic Fisheries Science* 3:41-45.

Okutani, T. 1983. *Todarodes pacificus. In: Cephalopod Life Cycles, Vol. 1,* P. R. Boyle, ed. pp. 201-214. Academic Press, London.

Sakurai, Y., M. Shimizu, Y. Ikeda, and K. Shimazaki. 1993. Feeding and growth of captive adult Japanese common squid, *Todarodes pacificus*, measuring initial body size by cold anesthesia. *In*: *Recent Advances in Cephalopod Fisheries Biology,* T. Okutani, R. K. O'Dor, and T. Kubodera, eds. pp. 467-476. Tokai University Press, Tokyo.

Sakurai, Y., R. E. Young, J. Hirota, K. Mangold, M. Vecchione, M. R. Clarke, and J. Bower. 1995. Artificial fertilization and development through hatching in the oceanic squids *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*. *The Veliger* 38(3):185-191.

Watanabe, K., Y. Sakurai, S. Segawa, and T. Okutani. 1996. Development of the ommastrephid squid *Todarodes pacificus,* from fertilized egg to rhynchoteuthion paralarva. *American Malacological Bulletin* 13(1/2):73-88.

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Systematics and biogeography of the genus *Donax* (Bivalvia: Donacidae) in eastern North America

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Abstract: Recent work with RAPD DNA markers (Adamkewicz and Harasewych, 1994) revealed the absence of fixed differences between the subspecies *Donax variabilis variabilis* Say, 1922, and *D. variabilis roemeri* Philippi, 1849, confirmed that *D. parvulus* Philippi, 1849, is distinct from *D. texasianus* Philippi, 1847, and established that *D. parvulus* is more closely related to *D. variabilis* than to *D. texasianus*, which it otherwise resembles. A fresh examination of RAPD markers in the remaining two Carolinian nominate species has shown that *D. fossor* Say, 1822, is indistinguishable from *D. parvulus* for all markers studied. As the name *D. fossor* has priority, *D. parvulus* should be considered its synonym. Similarly, *D. dorotheae* Morrison, 1971, was indistinguishable from *D. texasianus*, and should therefore be placed in the synonymy of *D. texasianus*. Thus, the biogeography of *Donax* is considerably simplified. *D. variabilis*, the larger, more intertidal species, occurs on both the Atlantic and Gulf coasts. Its division into two subspecies separated by the mouth of the Mississippi River cannot be justified based on RAPD data. *D. variabilis* shares each coast with a smaller, subtidal species, *D. fossor* on the Atlantic coast and *D. texasianus* in the Gulf of Mexico, with the Florida peninsula separating the subtidal species. We suggest that the emergence of Florida during the Cenozoic served as a barrier that led to the differentiation of *D. fossor* and *D. texasianus*. *D. variabilis* apparently evolved as an offshoot from *D. fossor* and subsequently entered the Gulf of Mexico, perhaps when it was connected to the Atlantic by the Suwanee Strait.

Key words: *Donax*, RAPD, systematics, biogeography, Carolinian Province

Determining the status of closely related taxa with allopatric distributions is always difficult and represents one of the most useful applications of molecular techniques to systematic problems. Having used randomly amplified polymorphic DNA (RAPD) markers to examine the relationships of sympatric pairs of *Donax* species (Adamkewicz and Harasewych, 1994), we next applied the technique to study allopatric species in the same complex. In his revision of the western Atlantic species of *Donax*, Morrison (1971) recognized six taxa, representing two adaptive strategies, as occurring along the Atlantic and Gulf coasts of North America (Fig. 1A). Two of these taxa, the subspecies *Donax variabilis variabilis* Say, 1822, and *D. variabilis roemeri* Philippi, 1849, display one adaptive strategy: they are fairly large (15-20 mm), with flattened, triangular shells, and occupy the middle intertidal zone, migrating actively with the tide. *D. variabilis* usually occurs sympatrically with a member of the second adaptive complex: these clams are smaller (5-8 mm), with smoother, more inflated shells, have an intertidal to subtidal distribution, and little tendency to migrate with the tide. Morrison recognized four allopatric species in this second group, referred to here as the subtidal species complex. These are *D. fossor* Say, 1822, which ranges from New Jersey to Cape Hatteras, *D. parvulus* Philippi, 1849, occurring from Cape Hatteras to eastern Florida, *D. dorotheae* Morrison, 1971, from the Gulf coast of Florida to Louisiana, and *D. texasianus* Philippi, 1847, reported from Louisiana to Vera Cruz, Mexico.

Distinguishing young, therefore small, specimens of *Donax variabilis* from any member of the subtidal species complex is quite difficult because the morphological distinctions are few and largely subjective (Morrison, 1971). This difficulty has led to controversies regarding the validity of several of the species in the subtidal species complex (Loesch, 1957; Chanley, 1969; Abbott, 1974) and prompted our original effort to find molecular markers that would resolve these questions (Adamkewicz and Harasewych, 1994). The RAPD molecular markers discovered in that study confirmed the distinctions between *D. variabilis* and two members of the subtidal species complex, and supported the validity of three of the North American taxa recognized by Morrison: *D. parvulus, D. texasianus*, and *D. variabilis.* However, no diagnostic markers were found to distinguish between the subspecies *D. variabilis variabilis* and *D. variabilis roemeri,* nor were any markers identified that were unique to either *D. parvulus* or *D. texasianus*. We now report the results of continuing research to further characterize these taxa using additional RAPD markers and to assess the relationships of *D. fossor* and *D. dorotheae*.

Data on the degree of differentiation among these donacid taxa can also shed light on the significance of

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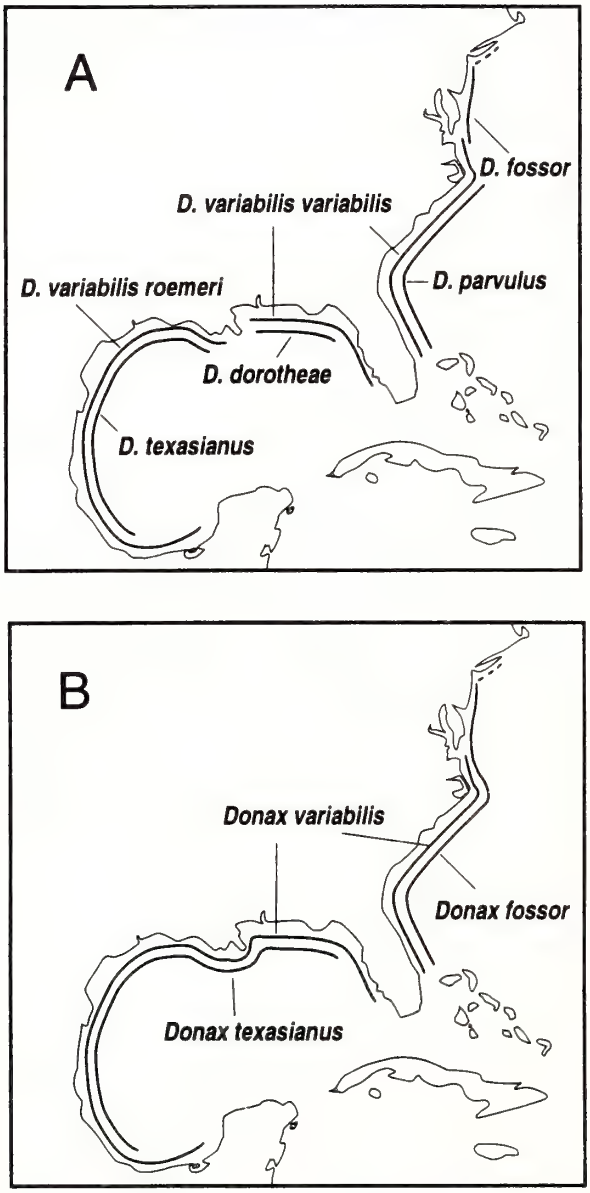

Fig. 1. A. Geographic distributions of the six *Donax* taxa recognized by Morrison (1971). **B.** Geographic distribution of *Donax* based on the findings of the present study.

major and minor geographic barriers along the coastal southeastern United States as isolating mechanisms for shoreline marine organisms with planktonic larvae. The mouths of two great rivers - the Chesapeake Bay complex and the Mississippi River - can create barriers to the dispersal of marine organisms, and were thought by Morrison (1971) to have a significant impact on the distribution of *Donax*. In his scheme (Fig. 1A), the northern limit of *D. variabilis variabilis* is the mouth of the Chesapeake, while Cape Hatteras, with its changes in currents, marks the boundary between *D. fossor* and *D. parvulus*. The mouth of the Mississippi River is the geographical boundary between *D. variabilis variabilis* and *D. variabilis roemeri* as well as between *D. dorotheae* and *D. texasianus*. Another major barrier, the peninsula of Florida, separates *D. parvulus* from *D. dorotheae*, and interrupts the distribution of *D. variabilis variabilis*.

The degree to which the Florida peninsula imposes a biogeographic boundary between the fauna of the southern Atlantic coast of the United States and the fauna of the northern Gulf of Mexico, both components of the Carolinian Province, has been a topic of considerable interest. Several molecular techniques have been used to assess genetic differentiation between Gulf and Atlantic populations of a variety of taxa. Restriction fragment length polymorphism (RFLP) analyses of the mitochondrial genomes of horseshoe crabs, toadfish, black sea bass, and diamondback terrapins have revealed significant differences in populations separated by the Florida peninsula (Avise, 1992), as did allozyme studies of a sea anemone (McCommas, 1982) and a marsh crab (Felder and Staton, 1994). Although no evidence of differentiation was found in allozyme studies of the oyster *Crassostrea virginica* (Gmelin, 1791) (see Buroker, 1983) or the periwinkle *Littorina irrorata* (Say, 1822) (see Dayan and Dillon, 1995), investigations of Atlantic and Gulf coast oyster populations using mitochondrial (Reeb and Avise, 1990) and nuclear (Karl and Avise, 1992) DNA, revealed genetic divergences.

MATERIALS AND METHODS

PREPARATION OF DNA SAMPLES

Specimens of *Donax fossor* were collected by one of us (MGH) in Wildwood Crest, New Jersey, immediately frozen and transported back to the laboratory at George Mason University where they were stored at -60°C. Specimens of *D. dorotheae* were collected from their type locality at Alligator Point, Florida, by Gulf Specimen Marine Laboratories, Inc. (P. O. Box 237, Panacea, Florida 32346) and shipped alive to the laboratory, where their identification was confirmed before they were stored at -60°C. Voucher material is deposited at the National Museum of Natural History, Smithsonian Institution (*D. fossor*, USNM 888672; *D. dorotheae*, USNM 888673). For collection localities and voucher information on the remaining species of *Donax* used in this study, see Adamkewicz and Harasewych (1994: table 1).

To obtain DNA from these specimens, foot muscle was dissected from thawed animals and extracted using a modification of the method of Doyle and Doyle (1987) communicated to us by Andrew McArthur, and further modified in our laboratory. This method has proven both simple and superior to most others in terms of the quality of the DNA produced. Approximately 100-600 µg of tissue was ground in 300 µl of heated (60°C) buffer composed of 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, and 2% Hexadecyltrimethylammonium bromide (CTAB). As a modification to prevent co-isolation of contaminants, we included 2% polyvinyl pyrolidone in the extraction buffer. Immediately prior to use, we added 0.2% mercaptoethanol to the buffer. The macerate was incubated at 60°C for at least 30 min, then shaken with 300 µl of chloroform/ isoamyl alcohol 24:1 and spun at 14,000 x g for 5 min. The upper, aqueous phase was transferred to a clean tube and treated again with chloroform/isoamyl alcohol. After the aqueous phase was again transferred to a clean tube, the DNA was precipitated by adding 25 µl of 3 M sodium acetate and 600 µl of 70% ethyl alcohol. The mixture was spun at 14,000 x g for 10 min and the supernatant discarded. The DNA pellet was washed twice in 300 µl of 70% ethyl alcohol and dried at 60°C before being dissolved in 50-100 µl of TE buffer (10 mM Tris, 1 mM EDTA) and stored frozen.

PRODUCTION OF RAPD MARKERS

Aliquots of DNA were amplified by the polymerase chain reaction (PCR) according to our earlier RAPD protocol (Adamkewicz and Harasewych, 1994: 53). When the amplification products were separated on a 1.4% agarose gel, this procedure produced DNA fragments of several sizes for each primer, the relative sizes being determined by comparison to DNA markers of known sizes run on the same gel. Our original study (Adamkewicz and Harasewych, 1994) screened 60 primers and found five that were informative about relationships among five species of *Donax*. The present study assayed 20 additional primers, of which one proved to be informative. With two exceptions, the 80 primers used in this study were obtained from Operon Technologies (1000 Atlantic Avenue, Alameda California 94501) and are identified by OP followed by the kit identification letter and number. The remaining two primers were synthesized by the Laboratory for Molecular Systematics, National Museum of Natural History, Smithsonian Institution, and are identified by LMS followed by their identification number.

In the original study, each primer was tested on three specimens of each taxon, and chosen for further use only if it met certain criteria for amplification quality, reproducibility, and distribution of markers among taxa. At that time, our screening criteria emphasized fragments that were shared by multiple taxa, and no primer was studied further unless it generated a marker that occurred in more than one sample of *Donax*. Therefore, taxon-specific markers were identified only for the two species, *D. variabilis* and *D. denticulatus* Linné, 1758, that were represented in our study by multiple populations. To remedy this bias, the present study sought to identify additional taxon-specific markers by several methods. First, we assayed nine individuals each of *D. fossor* and *D. dorotheae* for the 17 markers produced by the five primers identified in our previous study. In addition, we screened these new taxa plus *D. parvulus* and *D. texasianus* samples from our previous study for an additional 20 primers from Operon Kit A. The principle criterion in this screening was that a primer should produce a DNA fragment present in most or all members of one or more taxa. This search identified one new primer which was then used on all samples from the earlier study. Finally, we re-examined photographs of the screening gels from our original study in order to identify any primers that would have met this new standard. This search added two new markers from previously tested primers to the data set. The sequences of old and new primers, and the sizes of informative markers they produced, are summarized in Table 1.

Table 1. DNA primers used in this study. For each primer, the table shows its sequence and the sizes in kilobases of those DNA amplification products used as markers. Primers designated OP A and OP E are from Operon Technologies Kits A and E respectively. Primers designated LMS P were provided by the Laboratory for Molecular Systematics, National Museum of Natural History, Smithsonian Institution. Undesignated fragments are those used in our earlier study (Adamkewicz and Harasewych, 1994). Fragments marked with an asterisk (*) are newly reported in this study.

PRIMER	SEQUENCE	USEFUL RAPD MARKERS (sizes in kb)				
OP A07	5'-GAAACGGGTG	0.3*				
OP E07	5'-AGATGCAGCC	0.6	1.5			
OP E16	5'-GGTGACTGTG	0.3	0.5	0.6	0.9	1.1
OP E18	5'-GGACTGCAGA	0.5	0.6	0.9		
LMS P01	5'-TGGTCAGTGA	0.5	0.6*	1.0	1.2	2.0*
LMS P56	5'-AGATCTGCAG	0.3	0.6	1.1	1.2	

PHYLOGENETIC ANALYSES

Data from nine populations, including all six Recent nominal species and subspecies of Carolinian *Donax* were used in this study. A sample of *D. denticulatus* from Negril, Jamaica, and a sample of *D. striatus* Linné, 1767, from Black River, Jamaica, were used as outgroups, based on a previously published phylogeny (Adamkewicz and Harasewych, 1994). As in our earlier study, each RAPD marker was treated as a separate character without regard to the primer used to produce it or whether other bands produced by the same primer co-occurred with it. Markers were scored as absent (0), polymorphic (1), *i. e.* present in some, but not all, members of a population, or fixed (2), *i. e.* present in all members of a population. Maximum parsimony trees, bootstrap values (1000 replicates), and tree diagnostics were calculated using PAUP version 3.1 (Swofford, 1993). Characters were treated as ordered (0 ↔ 1 ↔ 2), because this models the way in which alleles enter, become established, and leave populations. Branch support values (*b*) and the total support index (*ti*) were calculated using procedures outlined by Bremer (1994: 300). Data were entered and character evolution on the resulting trees analyzed using MacClade version 3.01 (Maddison and Maddison, 1992).

RESULTS

RAPD DNA MARKERS

Both sets of screening criteria were successful in identifying taxon-specific RAPD markers among the *Donax* species of our original study. Of the new primers tested, one was found to produce a marker (OP A07 0.3 kb) unique to *D. texasianus* and *D. dorotheae*. A re-analysis of previous data revealed one marker (LMS P01 2.0 kb) unique to *D. parvulus* that was also found to occur in *D. fossor*, and another marker (LMS P01 0.6 kb) that was unique to *D. striatus*. Thus, each of the five species in our 1994 study can now be distinguished by the presence of one or more unique markers (Table 2). Of the 20 markers used in the present study, eight are common to all the Carolinian populations examined. Two are unique to *D. variabilis*, which shares another two markers with the two nominal, Atlantic, subtidal taxa, *D. fossor* and *D. parvulus*. The Atlantic *D. fossor/D. parvulus* pair and the Gulf coast *D. texasianus/D. dorotheae* pair are each distinguished by a unique marker. Although unique markers were sought for each taxon, none were found that would distinguish between the two members of either of these pairs.

Table 2. RAPD DNA markers diagnostic for species of northwestern Atlantic Donacidae. Markers preceded by a dagger (†) are fixed, the remaining markers are present in most but not all members of their respective taxa. See Table 3 for frequencies of markers.

TAXON	DIAGNOSTIC MARKER(S)
Donax fossor (+ *D. parvulus*)	LMS P01 2.0 kb
D. variabilis variabilis (+ *D. variabilis roemeri*)	OP E16 0.3 kb; †OP E18 0.5 kb
D. texasianus (+ *D. dorotheae*)	OP A07 0.3 kb
D. denticulatus	†OP E16 0.6 kb; OP E18 0.6 kb; LMS P01 1.0 kb
D. striatus	LMS P01 0.6 kb

The marker unique to the *Donax texasianus/D. dorotheae* pair, a *ca.* 300 bp fragment produced by primer OP A 07, was present in eight of nine *D. texasianus* individuals examined, as well as in six of nine *D. dorotheae* analyzed. Similarly, the 2 kb fragment produced by primer LMS P01 was present in all nine individuals of *D. parvulus* and in eight of nine individuals of *D. fossor*. After a screening with 80 primers and a detailed examination with six primers, only very minor differences in marker frequencies separated the members of these two pairs. Such differences have little meaning when sample sizes are small, nine individuals per taxon, and differences as large or larger were found among the species represented by multiple populations, *D. variabilis* and *D. denticulatus* (Table 3).

PHYLOGENETIC ANALYSIS

A single most parsimonious tree (length = 36, ci = ri = 0.92, ti = 0.42), shown in Fig. 2, was produced using the data matrix given in Table 4 and the Exhaustive Search command, which guarantees that all minimum length trees will be found (Fig. 2). Three additional steps would be required to unite the members of the subtidal species complex in a clade. Of the 20 markers, four (markers 14, 15, 17, and 20) were phylogenetically uninformative, appearing only in terminal taxa (autapomorphies). All but three of the remaining 16 markers had a consistency index of 1.0. Two of the exceptions (marker 6, ci = 0.667; 12, ci = 0.500), were apparent reversals from a fixed to a polymorphic state in the *D. fossor/D. parvulus* pair. The remaining exception (marker 10, ci = 0.667) was accounted for by an independent (homoplastic) fixation of the marker in *D. denticulatus* and in the *D. fossor/D. parvulus* pair.

DISCUSSION

The four nominal taxa of the subtidal species complex replace each other geographically, but neighboring taxa do not differ morphologically in any clearly recognizable way and all occupy the same ecological niche. Although the binomen *Donax fossor* is among the oldest to be applied to American donacids, the systematic relationships of this taxon are among the most poorly understood. Jacobson and Emerson (1961) noted the sporadic occurrence of these animals in Long Island, and suggested that this taxon represented juvenile or stunted specimens of *D. variabilis* that were recruited from larvae swept north of the sustainable range of this species. They further reported that *D. fossor* does not survive the winter in this area. Chanley (1969) reiterated this hypothesis on the basis of his studies of seasonal distribution of *Donax* in the mid-Atlantic region, and speculated that "minor conchological

Table 3. Distribution of the RAPD DNA markers among sample populations. For each RAPD marker, the band designation identifies the primer, the approximate size of the marker in kilobases and a sequential number to identify the marker. Each entry in the matrix shows the number of individuals in which the RAPD marker was detected over the number of individuals tested (*e. g.* 3/9). In a few cases, the number of individuals tested was fewer than nine either because fewer DNA samples were available or because we were unable to score an individual for a particular marker. When no individuals produced a marker, the entry is marked "–" rather than 0/n. Data are grouped to emphasize affinities among sample populations, rather than by primer. Abbreviations (DDB, DDM, DDN, DVF, DVG, DVR) as in Adamkewicz and Harasewych (1994).

	LMS	OP	OP	OP	LMS	OP	OP	LMS	LMS	OP	OP	OP	OP	OP	LMS	LMS	OP	LMS	LMS	LMS
PRIMER:	P01	E18	E16	E16	P56	E16	E16	P01	P56	E18	E07	E07	A07	E18	P01	P01	E16	P56	P56	P01
SIZE (kb):	2.0	0.5	0.3	0.9	0.3	0.5	1.1	0.5	0.6	0.9	0.6	.5	0.3	0.6	1.0	1.2	0.6	1.1	1.2	0.6
NUMBER:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Donax fossor	8/9	–	–	4/9	6/9	7/9	2/9	9/9	9/9	9/9	3/9	4/9	–	–	–	–	–	–	–	–
D. parvulus	9/9	–	–	7/9	6/9	7/9	2/9	9/9	9/9	9/9	2/9	6/9	–	–	–	–	–	–	–	–
D. v. variabilis (DVF)	–	9/9	2/9	5/9	8/9	9/9	3/9	9/9	9/9	9/9	9/9	9/9	–	–	–	–	–	–	–	–
D. v. variabilis (DVG)	–	9/9	4/9	5/9	9/9	9/9	49	9/9	9/9	9/9	9/9	9/9	–	–	–	–	–	–	–	–
D. v. roemeri (DVR)	–	9/9	1/9	5/9	6/9	9/9	6/9	9/9	9/9	9/9	9/9	9/9	–	–	–	–	–	–	–	–
D. dorotheae	–	–	–	–	–	7/7	7/7	4/9	9/9	2/9	1/9	9/9	6/9	–	–	–	–	–	–	–
D. texasianus	–	–	–	–	–	9/9	9/9	3/9	9/9	2/9	2/9	9/9	8/9	–	–	–	–	–	–	–
D. denticulatus (DDB)	–	–	–	3/3	–	–	3/3	–	–	3/3	2/3	2/3	–	3/3	3/3	1/3	2/3	3/3	1/3	–
D. denticulatus (DDM)	–	–	–	9/9	–	–	9/9	–	–	9/9	5/9	2/9	–	9/9	8/9	1/9	8/9	6/6	5/8	–
D. denticulatus (DDN)	–	–	–	9/9	–	–	9/9	–	–	9/9	7/9	7/9	–	9/9	9/9	1/9	8/9	5/9	6/9	–
D. striatus	–	–	–	6/6	–	–	6/6	–	–	–	–	5/6	–	–	–	6/6	–	4/6	6/6	8/9

Table 4. Data matrix for cladistic analyses. Markers listed in Table 2 are scored as: absent (0), polymorphic (1), fixed (2), or unknown (?). Abbreviations as in Table 3.

MARKER #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Donax fossor	1	0	0	1	1	1	1	2	2	2	1	1	0	0	0	0	0	0	0	0
D. parvulus	2	0	0	1	1	1	1	2	2	2	1	1	0	0	0	0	0	0	0	0
D. v. variabilis (DVF)	0	2	1	1	1	2	1	2	2	2	2	2	0	0	0	0	0	0	0	0
D. v. variabilis (DVG)	0	2	1	1	2	2	1	2	2	2	2	2	0	0	0	0	0	0	0	0
D. v. roemeri (DVR)	0	2	1	1	1	2	1	2	2	2	2	2	0	0	0	0	0	0	0	0
D. dorotheae	0	0	0	0	2	2	1	2	1	1	2	1	0	0	0	0	0	0	0	0
D. texasianus	0	0	0	0	0	2	2	1	2	1	1	2	1	0	0	0	0	0	0	0
D. denticulatus (DDN)	0	0	0	2	0	0	2	0	0	2	1	1	0	2	2	1	1	1	1	0
D. striatus	0	0	0	2	0	0	2	0	0	0	0	1	0	0	0	2	0	1	2	1

differences" between *D. fossor* and *D. variabilis* were likely ecophenotypic. Most (*e. g.* Abbott, 1974; Emerson and Jacobson, 1976), but not all (Morrison, 1971) subsequent workers have treated *D. fossor* as a synonym of *D. variabilis*. *D. parvulus* was also regarded as an offshore ecological form of *D. variabilis* by some authors (*e. g.* Abbott, 1974).

Implicit in the hypotheses of seasonal northern range extensions (in the case of *Donax fossor*) or offshore ecophenotypes (in the case of *D. parvulus*) is the assumption that these populations comprise subsets of the genetic variation to be found in *D. variabilis*. Our RAPD data demonstrate clearly that, although *D. fossor* and *D. parvulus* cannot be distinguished from each other, both are genetically distinct from *D. variabilis*. Because the ranges of *D. fossor* and *D. parvulus*, as suggested by Morrison (1971), are allopatric, it is difficult to be certain that this indistinguishable pair is, in fact, the same species. However, a careful search for significant differences in their gene pools has produced entirely negative results. As *D. fossor* is the older name, it should be applied to all members of the subtidal species complex living along the Atlantic coast of the United States, and *D. parvulus* treated as its synonym. This taxon is characterized by the presence of a 2.0 kb marker produced by the LMS P01 primer in most members of its populations. Our coarse sampling of two populations from near the extremes of the range of this species failed to uncover differences that might justify even a subspecific distinction of these taxa.

Similarly, patterns of RAPD markers in *Donax dorotheae* do not differ in any meaningful way from those in *D. texasianus*. Only the most minor differences in frequencies of polymorphic markers were found, and these were well within the range of inter-population variation in *D. variabilis*. The name *D. texasianus* has priority, and should be applied to all Gulf coast members of the subtidal species complex. *D. texasianus* may be diagnosed by the presence, in the majority of the individuals in its populations, of a 0.3 kb marker generated by the OP A07 primer.

Additional data from the present study have failed to support the subspecific division of *Donax variabilis* that Morrison suggested. Differences between the Atlantic and eastern Gulf populations appear to be of similar type and magnitude to those between the eastern and western Gulf populations, and are within the range of variation of three populations of *D. denticulatus* from around Jamaica (Table 3), as well as of a single population of *D. denticulatus* sampled at different times (Adamkewicz and Harasewych, 1994: table 3).

If the above changes are adopted, the distribution of *Donax* along the Atlantic and Gulf coasts of North America changes from that proposed by Morrison (Fig. 1A) to the one shown in Fig. 1B.

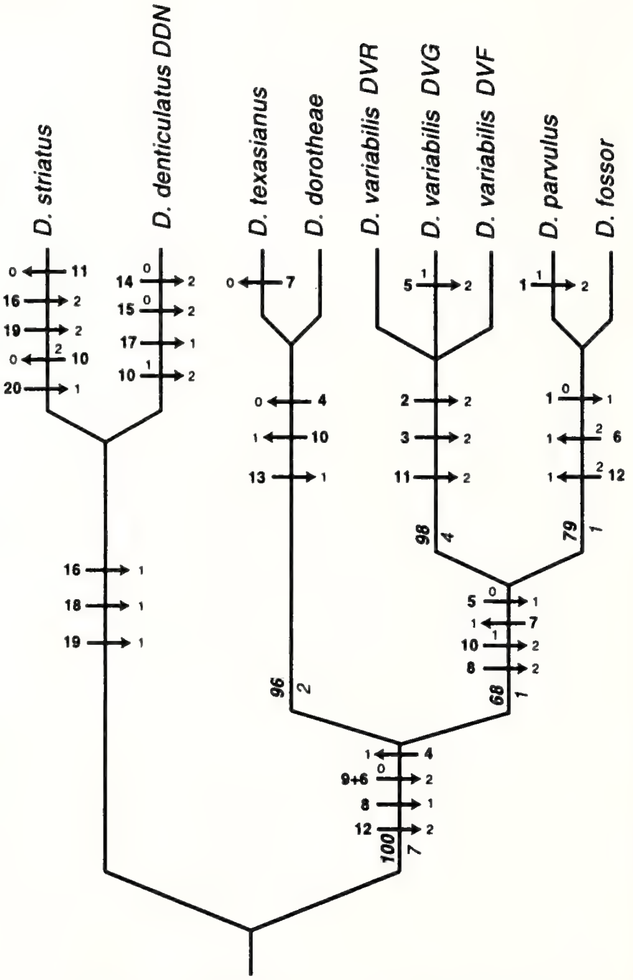

Fig. 2. Phylogenetic relationships of the six *Donax* taxa recognized by Morrison (1971) and two additional *Donax* taxa identified as outgroups by Adamkewicz and Harasewych (1994). Relationships are inferred from the distribution of the 20 RAPD markers shown in Table 3.

The inclusion of additional taxa and characters in our data matrix produced a single most parsimonious tree (Fig. 2) identical in topology to that from our previous study (Adamkewicz and Harasewych, 1994: figs 2-3). The topology of the phylogenetic tree indicates that the small size and non-migratory subtidal habitat of *Donax fossor* and *D. texasianus* are ancestral conditions, while the larger size, elongated shell morphology and migratory, intertidal habitat of *D. variabilis* are more recently evolved. The difficulty in discriminating juvenile *D. variabilis* from *D. fossor* using morphological characters, and the resulting confusion regarding their taxonomy, are likely a consequence of the sister-group relationship between these taxa. *D. variabilis*, the younger species, retains the morphology of its smaller ancestors at a comparable body size but, as an adult, develops a larger size and more derived shell form, possibly adapted to its migratory habitat.

Of particular interest is the lack of correspondence in the distributions and biogeographic boundaries of *Donax variabilis* and the members of the subtidal species complex. Peninsular Florida separates the Atlantic and Gulf species of the subtidal complex but does not appear to have caused discernible differentiation in populations of *D. variabilis. D. fossor* inhabits the Atlantic coast of the United States from the extreme north of the range for any donacid species to central Florida. Jacobson and Emerson (1961) and Chanley (1969) were likely correct in their conjecture that northernmost populations of *D. fossor* are ephemeral range extensions during seasonally favorable conditions of a species with a more southern distribution, but erred in regarding them to be conspecific with *D. variabilis*. Our admittedly coarse samples, which span both Chesapeake Bay and Cape Hatteras, reveal few differences between populations and suggest that neither of these geographical features are barriers to gene flow in *D. fossor*. Similarly, our samples of *D. texasianus*, the Gulf member of the subtidal complex, span the Mississippi Delta and also demonstrate the absence of any barrier to gene flow within the Gulf of Mexico. For this genus, the major rivers apparently do not impede gene flow. In contrast, the Florida peninsula separates *D. fossor* and *D. texasianus* but has not produced qualitative differences among populations of *D. variabilis*, which it also separates. Because studies surveying variation in the mitochondrial genome have been most successful in discovering genetic differentiation between Gulf and Atlantic conspecific populations (Avise, 1992), perhaps the application of such techniques would reveal differentiation among populations of *D. variabilis*. Our RAPD study does not do so.

The different ages of these taxa could contribute to the differences in their geographic distributions. The emergence of peninsular Florida could have contributed to the vicariant differentiation of *Donax fossor* and *D. texasianus*. *D. variabilis* appears to have evolved from *D. fossor* along the Atlantic coast, although by what mechanism we have no evidence. *D. variabilis* could then have invaded the Gulf of Mexico through the Suwanee Straits (Huddlestun *et al.*, 1988).

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LITERATURE CITED

Abbott, R. T. 1974. *American Seashells, 2nd edition.* Van Nostrand Reinhold Co., New York. 663 pp.

Adamkewicz, S. L. and M. G. Harasewych. 1994. Use of randomly amplified polymorphic DNA (RAPD) markers to assess relationships among beach clams of the genus *Donax. The Nautilus* supplement 2:51-60.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of regional fauna: a case history with lessons for conservation biology. *Oikos* 63:62-76.

Bremer, K. 1994. Branch support and tree stability. *Cladistics* 10:295-304.

Buroker, N. E. 1983. Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and Gulf of Mexico. *Marine Biology* 75:99-112.

Chanley, P. 1969. *Donax fossor*: a summer range extension of *Donax variabilis. The Nautilus* 83(1):1-14.

Dayan, N. S. and R. T. Dillon, Jr. 1995. Florida as a biogeographic boundary: evidence from the population genetics of *Littorina irrorata. The Nautilus* 108(3):49-54.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19:11-15.

Emerson, W. K. and K. M. Jacobson. 1976. *The American Museum of Natural History Guide to Shells, Land, Freshwater, and Marine, from Nova Scotia to Florida.* Alfred A. Knopf, New York. 482 + xviii pp., 47 pls.

Felder, D. L. and J. L. Staton. 1994. Genetic differentiation in trans-Florida species complexes of *Sesarma* and *Uca* (Decapoda: Brachyura). *Journal of Crustacean Biology* 14:191-209.

Huddlestun, P. F., M. E. Hunter, and B. D. Carter. 1988. The Suwanee Strait as a faunal provincial boundary. *The Geological Society of America, Abstracts with Programs* 20(4):271.

Jacobson, K. M. and W. K. Emerson. 1961. *Sea Shells of the New York City Area.* Argonaut Books, Larchmont, New York. 142 pp.

Karl, S. A. and J. C. Avise. 1992. Balancing selection at allozymic loci in oysters: implications from nuclear RFLPs. *Science* 256:100-102.

Loesch, H. C. 1957. Studies on the ecology of two species of *Donax* on Mustang Island, Texas. *Publication of the Institute of Marine Science, University of Texas* 4:201-227.

Maddison, W. P. and D. R. Maddison. 1992. *MacClade, Analysis of Phylogeny and Character Evolution, Version 3.* Sinauer Associates, Sunderland, Massachusetts.

McCommas, S. A. 1982. Biochemical genetics of the sea anemone *Bunodosoma cavernata* and the zoogeography of the Gulf of Mexico. *Marine Biology* 68:169-173.

Morrison, J. P. E. 1971. Western Atlantic *Donax. Proceedings of the Biological Society of Washington* 83(48):545-568, 2 pls.

Reeb, C. A. and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica. Genetics* 124:397-406.

Swofford, D. L. 1993. *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.* Illinois Natural History Survey, Champaign, Ilinois.

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Effects of temperature on byssal thread production by the freshwater mussel, *Dreissena polymorpha* (Pallas)

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Abstract: Byssal thread production by specimens of *Dreissena polymorpha* (Pallas, 1771) was monitored at water temperatures of 5°, 10°, 15°, 25° and 30°C following re-attachment. Incremental increases in byssal thread number were recorded and the mean number of byssal threads produced by 20 mussels at each temperature calculated over a 21 day period. Rate of byssal thread production increased proportionally with increasing temperature, the slowest rate being at 5°C, the highest rate at 30° C, just 1°C below the reported upper lethal temperature for this species. A significantly greater number of byssal threads were formed by mussels exposed to high test temperatures (15°, 25° and 30°C) than those exposed to low temperatures (5° and 10°C). At 30°C, byssus re-formation appeared to consist of two phases, an initial phase of rapid thread production (1-7 days), followed by a phase in which production rate slowed (8-21 days). At low temperatures, there was no evidence of a two phase re-formation of the byssal mass within the experimental time period, the rate of thread production being constant throughout. This suggests that the length of the initial phase of rapid byssal thread production is temperature dependent. Mussels maintained at lower temperatures could have remained in the initial phase of byssal mass re-formation throughout the 21 day experimental period. Alternatively, mussels held at low temperatures could produce a mature byssus containing significantly fewer byssal threads than those exposed to higher environmental temperatures.

Key words: zebra mussels, *Dreissena*, byssal threads, temperature

The zebra mussel, *Dreissena polymorpha* (Pallas, 1771) is a freshwater bivalve mollusk, recently introduced into North America from Europe (Roberts, 1989). *D. polymorpha* utilizes a byssal hold-fast to secure to hard surfaces. Attachment is achieved by the production of proteinaceous 'byssal threads' from a gland at the base of the mussel's foot (Morton, 1969). Byssal glands are present in the late larval and post-larval stages of most epifaunal bivalve mollusks and function to allow stable attachment of the larva (*i. e.* the pediveliger) to hard substrata during metamorphosis into the juvenile (*i. e.* the plantigrade). Marine mytilids and freshwater dreissenids are two of the few groups of bivalves that neotenously retain byssal attachment beyond the juvenile stage (Yonge, 1962). Retention of byssal threads by the adult is fundamental to the success of these two groups as colonizers of hard substrata and as macrofoulers of man-made raw-water structures and systems (Mackie *et al.*, 1989).

Seasonal variations in byssal attachment strength of the intertidal marine bivalve, *Mytilus edulis* Linné, 1758, have been attributed to a combination of environmental variables. Water temperature, wave exposure, and current flow are among the seasonal variables affecting number of byssal threads produced (Price, 1980). Several laboratory investigations of the effects of temperature on byssal thread production by *M. edulis* and *Geukensia demissa* (Dillwyn, 1817) have been performed (Glaus, 1968; van Winkle, 1970; Allen *et al.*, 1976; Price, 1982; Young, 1985). However, the results of these studies are somewhat inconclusive; some studies suggested that elevated temperatures stimulate increased rate of byssal thread production by *M. edulis* (Glaus, 1968; Allen *et al.*, 1976; Young, 1985), while others indicated that either a negative relationship (van Winkle, 1970) or no relationship (Price, 1982) exists between byssal production rate and elevations in temperature.

Due to fundamental differences in ecology, evolution and taxonomy, it cannot be assumed that responses of the physiological processes regulating byssal thread production, to varying temperatures, will be similar for marine mytilid and freshwater dreissenid species (Clarke and McMahon, 1995). Although several studies of the biomechanical and morphological properties of zebra mussel byssal threads have been performed (Ackerman *et al.*, 1993a, b; Eckroat *et al.*, 1993), the effect of temperature on byssal thread production by dreissenids has not been addressed in a comparable manner to experiments carried out on mytilids. This study tests the hypothesis that the rate and number of byssal threads produced by *Dreissena polymorpha* will be temperature dependent, with byssal thread production rate increasing with elevated temperature.

METHODS

Specimens of *Dreissena polymorpha* were collected from the guide wall of Black Rock Lock on the Niagara

River, Buffalo, New York, on 25 February 1994 and 10 November 1994. Collected mussels were transported by overnight delivery service to the University of Texas at Arlington. Mussels were maintained in a 280 l, 'Living Stream' holding tank in aerated, dechlorinated water at a constant temperature of 5°C, without feeding, until utilized in experiments. Specimens have been maintained under these conditions with little mortality or tissue mass loss, for over one year (Chase and McMahon, 1994). Any specimens of 'Quagga mussels' (*Dreissena bugensis* Andrusov, 1897) found contaminating the sample were visually identified (May and Marsden, 1992) and removed. All experimental mussels were utilized within 90 days of collection.

Prior to experimentation, mussels were acclimated in water-filled aquaria at a test temperature of 5°, 10°, 15°, 25°, or 30°C for two weeks. During both the acclimation and experimental periods, constant water temperatures (± 0.1°C) were maintained by holding the aquaria in refrigerated incubators. After the acclimation period, all byssal threads were removed from the mussels by severing threads at the byssal gape with a razor blade. Immediately after removal of the byssus, mussels were allowed to byssally reattach overnight to 14 x 14 x 0.2 cm, clear, plexiglass plates placed in the bottom of the aquaria. The range of byssal thread production by individual mussels during this 12 h reattachment period was 2-29 threads/mussel. Thereafter, the cumulative number of threads produced by re-attaching mussels held at specific temperatures was recorded daily over a 21 day period following initial re-attachment (*i.e.* day 0).

Newly produced byssal threads were counted by viewing the underside of the mussel through the clear plexiglass plate under a dissecting microscope at 45x. The sites of thread attachment to the plate (*i. e.* the plaques) were clearly visible when viewed in this manner. The locations of new plaques were marked each day on the underside of the plate with a fine tipped, permanent marker. This allowed daily increments in newly formed byssal threads to be accurately counted. Mussels were fed with rehydrated, washed, freeze-dried green algae, *Chlorella* sp., in recommended quantities (*i. e.* 0.0032g/mussel/day; Nichols, 1993). Tank media was changed every two days.

After 21 days, mussels were removed from the plexiglass plate by carefully cutting the byssal attachment plaques with a razor blade. The shell length of each individual was measured to the nearest 0.1 mm with dial calipers (SL = greatest linear dimension from the dorsal to ventral shell margin). The mean SL (± s.d.) of mussels used in the study was 20.9 ± 2.5 mm (range 16.1 - 25.1 mm) at an experimental temperature of 5°C, 19.2 ± 3.4 mm (range 13.8 - 26.7 mm) at 10°C, 23.6 ± 2.2 mm (range 19.9 - 27.2 mm) at 15°C, 20.9 ± 3.6 mm (range 13.9 - 27.2 mm) at 25°C, and 22.4 ± 3.6 mm (range 16.8 - 30.0 mm) at 30°C.

This procedure was repeated for 20 individual mussels at each of the five test temperatures. Some mussels died or detached from the byssus before the 21 day test period elapsed (detachment from the byssus followed by relocation is common behavior for this species; Mackie *et al.*, 1989). Only those individuals which remained alive and attached throughout the entire 21 day test period were used in calculating mean daily byssal thread production (total N = 20 x 5 = 100).

As this experiment involved the repeated testing of individual mussels over time, an Analysis of Variance (ANOVA) with time (days) as a repeated measure was employed to analyze the data. With temperature as the main treatment, number of byssal threads produced by a mussel on first day of re-attachment (*i.e.* byssal thread number at day 0) and mussel shell length (SL) as covariates, the effect of temperature on byssal thread production over both 11 and 21 days exposure was analyzed. As the mean SL of the mussels used in each treatment was very similar (*i. e.* range of mean SL = 19.2-23.6 mm) and as the effect of SL was treated as a covariate in the ANOVA, differences in SL of individual mussels was not considered to be a confounding element in this statistical analysis.

RESULTS

The repeated measures ANOVA indicated that variations in temperature had a significant effect on the rate of byssal thread production by specimens of *Dreissena polymorpha* over both the initial 11 day and total 21 day exposure periods. The number of byssal threads produced differed significantly with the repeated measure factor of days (Table 1); significant interaction between temperature and days was present in both analyses (Table 1), suggesting that at some, or all, of the test temperatures, mussels produced byssal threads at differing rates throughout the exposure period.

Examination of the cumulative mean number of byssal threads produced over 21 days exposure indicated that, at 30°C, the rate of byssal thread production was highest between days 1 and 7, followed by a reduced rate between days 8 and 21 (Fig. 1). At the lower test temperatures of 5°, 10° and 15°C the rate of byssal thread production appeared to be relatively constant throughout the 21 day exposure period (Fig. 1). A post-hoc Least Significant Difference (LSD) test was performed to determine temperature treatment differences in byssal thread production rates both half way through (11 days) and at the end of the experimental period (21 days) (Table 2). Over 11 days

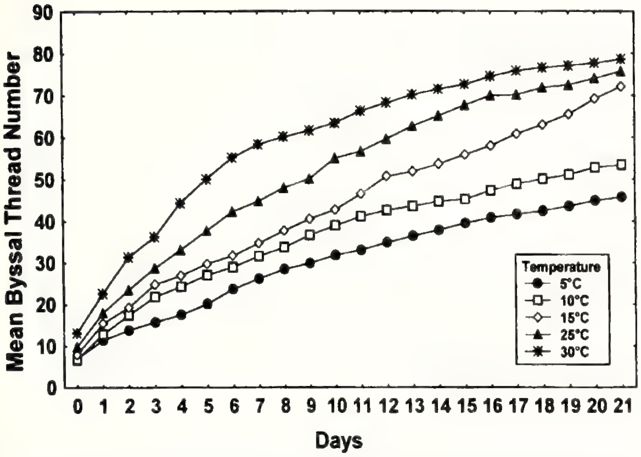

Fig. 1. Cumulative mean number of byssal threads produced by samples of 20 specimens of *Dreissena polymorpha* each day over a 21 day period, when exposed to water temperatures of 5°, 10°, 15°, 25°, and 30°C.

exposure there was a significantly higher byssal thread production rate at 30°C than at 25°C, however, there was no significant difference in byssal thread production rates between any other pairs of sequentially adjacent test temperatures (Table 2). As the experimental period approached 21 days, the 25°C and 30°C byssal thread production curves converged (Fig. 1). Consequently, over both 11 and 21 days exposure, the majority of significant differences in byssal thread production rates occurred between sequentially non-adjacent test temperatures (Table 2, Fig. 1).

After 21 days exposure, specimens of *Dreissena polymorpha* at 5°C had a mean byssal thread number of 45.8 ± 17.0 (s.d.) in their byssal mass, at 10°C a mean of 53.4 ± 25.1 threads/mass, at 15°C a mean of 72.1 ± 24.4 threads/mass, at 25°C a mean of 75.6 ± 36.4 threads/mass, and at 30°C a mean of 78.6 ± 32.9 threads/mass. A one-way Analysis of Variance followed by an LSD test showed that significantly fewer byssal threads were ultimately produced by mussels after 21 days exposure to 5° and 10°C than by those exposed to 15°, 25° or 30°C (Table 3).

DISCUSSION

Our results confirmed the hypothesis that temperature would significantly affect byssal thread production rate by the zebra mussel, *Dreissena polymorpha*, and may influence the ultimate number of byssal threads found in the mature byssal mass. At low temperatures (5° and 10°C), the pattern of cumulative byssal thread production was approximately linear, indicating a constant rate of byssal thread production over the 21 day exposure period. In contrast, at higher temperatures (25° and 30°C), cumulative byssal thread production became progressively curvilinear, suggesting two alternate phases of byssal thread production. There appears to be an initial phase of rapid byssal thread production followed by a second phase during which the rate of production is reduced, producing curvilinearity which is most pronounced at 30°C. The curvilinear response of byssal thread production was similar to that described by Paul (1980) for newly settled spat of the scallop *Aequipecten opercularis* (Linné, 1758). Spat of this species byssally attached more rapidly (and presumably produced byssal threads at a faster rate) as temperature was increased from 9° to 15° through 21°C. In addition, as we recorded for *D. polymorpha*, cumulative byssal thread production in *A. opercularis* spat became increasingly curvilinear at higher test temperatures (Paul, 1980). A similar, two-phase pattern of byssal mass re-formation has been described for the blue mussel, *Mytilus edulis*, the initial phase being characterized by an accelerated rate of thread formation and rapid accumulation of threads in the byssal mass; during the second phase thread production rate slowed as the mature byssal mass was maintained (Mahéo, 1970).

Although there were significant differences in the mean rate of byssal thread production by specimens of *Dreissena polymorpha* exposed to varying temperatures throughout the exposure period, there was no significant difference in the ultimate number of byssal threads found in the mature byssal mass of mussels exposed to test temperatures of 15°, 25°, or 30°C. The maximal, chronic upper lethal temperature of *D. polymorpha* is 31°C (McMahon *et al.*, 1994); byssal thread production was not suppressed at high environmental temperatures approaching this lethal limit. In contrast, at temperatures of 5° and 10°C, specimens of *D. polymorpha* produced significantly fewer byssal threads after 21 days exposure than did specimens at higher temperatures. Further, the cumulative number of byssal threads produced at lower test temperatures did not appear

Table 1. Repeated measures ANOVA examining the main effect of temperature (°C) with time (days) as the repeated measure, on byssal thread production rate by *Dreissena polymorpha* over 11 and 21 days of exposure to varying temperatures.

	1 to 11 Days Exposure			
Effect	df Effect	MS Effect	F	P
A) Temperature	4	8866	4.78	0.0015*
B) Days	10	11353	260	0.0001*
A x B Interaction	40	194	4.44	0.0001*
	1 to 21 Days Exposure			
A) Temperature	4	24067	3.69	0.0077*
B) Days	20	21477	299	0.0001*
A x B Interaction	80	260	3.61	0.0001*

* indicates a significant difference at level indicated

Table 2. LSD range test for significant differences in mean rate of byssal thread production between specimens of *Dreissena polymorpha* exposed to temperatures of 5°, 10°, 15°, 25°, and 30°C, over 11 and 21 days.

(°C)	1 to 11 days Temperature (°C)				1 to 21 days Temperature (°C)			
	10	15	25	30	10	15	25	30
5	0.167	0.032*	0.001*	0.001*	0.252	0.014*	0.001*	0.001*
10		0.432	0.008*	0.001*		0.183	0.005*	0.001*
15			0.055	0.001*			0.135	0.004*
25				0.015*				0.162

* indicates a significant difference at level indicated

to converge in the latter stages of the exposure period as it did at 15°, 25°, and 30°C. Byssal thread production at 5° and 10°C occurred at a constant, but reduced rate, that did not change greatly over the 21 day period, as was the case at higher test temperatures. The reduction of byssal thread production rate at the 5° and 10°C treatments may have resulted from a low temperature induced depression in metabolic rate, as oxygen consumption rates in *D. polymorpha* at 5°- 10°C are 4-6 fold lower than at 25°-30°C regardless of acclimation temperature (Mikheev, 1964; Lyashenko and Karchenko, 1989). The slower rate of byssal thread production by mussels exposed to low temperatures, and the fact that the 5° and 10°C curves do not converge in the latter stages of the exposure period as they do at the higher temperatures, suggests that they will ultimately form a byssal complex containing significantly fewer byssal threads than mussels at the high temperatures. Alternatively, at low temperatures mussels could take considerably longer to form a mature byssal holdfast, containing the same number of threads, than mussels at higher temperatures.

Table 3. ANOVA and LSD range test for total number of byssal threads present in the byssal complex of specimens of *Dreissena polymorpha* following 21 days of exposure to temperatures of 5°, 10°, 15°, 25°, and 30°C.

ANOVA

Source	Sum of Squares	df	Mean Square	F	p
Temperature	7876	4	1969	2.7	0.0348*
Error	67602	93	727		

LSD

Temperature (°C)	10	15	25	30
5	0.372	0.003*	0.001*	0.001*
10		0.031*	0.010*	0.004*
15			0.669	0.448
25				0.739

*indicates a signifiant difference at level indicated

Similar studies of the effect of temperature on byssal thread production by the marine mussel *Mytilus edulis*, have yielded a variety of results. Van Winkle (1970) reported almost total inhibition of byssal thread formation by this species at temperatures approaching 26°C, the upper lethal limit for *M. edulis* being 27°C (Hutchins, 1947). However, holding at 26°C produced no reduction in the rate of byssal thread production by *Geukensia demissa* (*fide* van Winkle, 1970) which has an upper thermal limit of 36° to 38°C (Lent, 1968). Similarly, Stern and Achituv (1978) reported a reduction in byssal thread production of 35% by the marine mytilid bivalve *Brachidontes variabilis* (Krauss, 1849) four days after being transferred from sea water (salinity 42 ppt) at 21°C to sea water at 27°C, with production falling to nearly zero four days after transfer to 33°C (41ppt), in which 100% mortality occurred after 38 days.

Our results indicate that maximal byssal thread production by *Dreissena polymorpha* occurred at 1°C below this species' upper thermal limit of 31°C (McMahon *et al.*, 1994). Similarly, Young (1985) reported a maximal rate of byssal thread production by specimens of *Mytilus edulis* held at 25°C, just two degrees below this species' upper thermal limit. Glaus (1968) also reported an increase in number of byssal threads produced by specimens of *M. edulis* with increasing exposure to temperatures of 18°, 23°, and 28°C. The rapid production of byssal threads reported at 28°C is interesting as it is 1°C above the reported long-term upper thermal limit of 27°C for *M. edulis* (*fide* Glaus, 1968). This suggests that, as we recorded for *D. polymorpha,* temperature stress does not appear to inhibit the byssal attachment mechanism of *M. edulis.*

CONCLUSIONS

Rate of byssal thread production by re-attaching adult specimens of *Driessena polymorpha* was temperature dependent, with production rate increasing progressively with increasing environmental temperature. At 30°C, the pattern of byssal mass re-formation was curvilinear and appeared to consist of an initial phase, lasting approximately seven days and being characterized by an accelerated byssal thread production rate. This was followed by a second phase during which byssal thread production rates were reduced. A similar two-phase pattern of byssus re-formation has also been described for *Mytilus edulis* (*fide* Mahéo, 1970). At lower temperatures the rate of byssal thread production by *D. polymorpha* was constant throughout the experimental period with no evidence of alternate phases. This could suggest that the time an individual mussel spends in the initial phase of byssal mass re-formation is temperature dependent, and given enough time mussels at lower temperatures will produce a mature byssus with thread numbers comparable to specimens exposed to higher temperatures. Alternatively, mussels at low temperatures could produce a mature byssal mass containing significantly fewer byssal threads than warm-water individuals; however, as the byssal holdfast must endure forces resulting from water movements, the magnitude of which are temperature independent, it would seem mal-adaptive for the zebra mussel to produce a compromised byssal attachment in a cold-water environment.

Elevated rates of byssal thread production were recorded at temperatures close to the zebra mussel's chronic upper thermal limit, indicating that temperature stress does not inhibit the physiological processes regulating byssal production in this species.

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Donna Urbanek of the Center for Biological Macrofouling Research, The University of Texas at Arlington, provided valuable technical assistance. Gary Dye, Lock Master at the U. S. Army Corps of Engineers Black Rock Lock, Buffalo, New York, provided experimental specimens. This *Dreissena polymorpha* research has been conducted as part of the Zebra Mussel Research Program of the United States Army Corps of Engineers under contract to the Center for Biological Macrofouling Research at the University of Texas at Arlington. Permission was granted by the Chief of Engineers to publish this information.

LITERATURE CITED

Ackerman, J. D., C. R. Ethier, D. G. Allen, and J. K. Spelt. 1993a. The biomechanics of byssal adhesion in *Dreissena polymorpha. In: Zebra Mussels: Biology, Impact, and Control*, T. F. Nalepa and D. W. Schloesser, eds. pp. 265-282. Lewis Publishers, Boca Raton, Florida.

Ackerman, J. D., C. M. Cottrell, C. R. Ethier, D. G. Allen, and J. K. Spelt. 1993b. The adhesion strength of zebra mussels: wall jet and direct mechanical tests. *In: Proceedings of the Third International Zebra Mussel Conference 1993.* pp. 2-79 - 2-92. EPRI TR-102077, Electric Power Research Institute, Palo Alto, California.

Allen, J. A., M. Cook, D. J. Jackson, S. Preston, and E. M. Worth. 1976. Observations on the rate of production and mechanical properties of the byssus threads of *Mytilus edulis* L. *Journal of Molluscan Studies* 42:279-289.

Chase, R. and R. F. McMahon. 1994. Effects of starvation at different temperatures on dry tissue and dry shell weights in the zebra mussel, *Dreissena polymorpha* (Pallas). *In: Proceedings of the Fourth International Zebra Mussel Conference 1994.* pp. 501-514. Wisconsin Sea Grant Institute, Madison, Wisconsin.

Clarke, M., and R. F. McMahon. 1995. Comparison of byssal attachment in dreissenid and mytilid mussels: mechanisms, morphology, secretion, biochemistry, mechanics and environmental influences. *Malacological Review* 28:25-40.

Eckroat, L. R., E. C. Masteller, J. C. Shaffer, and L. M. Steel. 1993. The byssus of the zebra mussel *(Dreissena polymorpha)*: morphology, byssal thread formation, and detachment. *In: Zebra Mussels: Biology, Impact, and Control,* T. F. Nalepa and D. W. Schloesser, eds. pp. 239-263. Lewis Publishers, Boca Raton, Florida.

Glaus, K. J. 1968. Factors influencing the production of byssus threads in *Mytilus edulis* (abstract). *Biological Bulletin* 135:420 .

Hutchins, L. W. 1947. The bases for temperature zonation in geographical distribution. *Ecological Monographs* 17:325-335.

Lent, C. M. 1968. Air-gaping by the ribbed mussel, *Modiolus demissus* (Dillwyn): effect and adaptive significance. *Biological Bulletin* 134:60-73.

Lyashenko, A. V. and T. A. Karchenko. 1989. Annual dynamics of energy metabolism in *Dreissena. Hydrobiologia* 25:34-41.

Mackie, G. L., W. N. Gibbons, B. W. Muncaster, and I. M. Gray. 1989. *The Zebra Mussel,* Dreissena polymorpha: *a Synthesis of European Experiences and Review for North America.* Great Lakes Section, Water Resources Branch, Ontario Ministry of the Environment, Queens Printer, Toronto, Ontario, Canada. 76 pp.

Mahéo, R. 1970. Étude de la pose et de l'activité de sécrétion du byssus de *Mytilus edulis* L. *Cahiers de Biologie Marine* 11:475-483.

May, B. and J. E. Marsden. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 1501-1506.

McMahon, R. F., M. A. Matthews, T. A. Ussery, R. Chase, and M. Clarke. 1994. Further studies of heat tolerance in zebra mussels: effects of temperature acclimation and chronic exposure to lethal temperatures. *In: Proceedings Fourth International Zebra Mussel Conference 1994.* pp. 251-272. University of Wisconsin Sea Grant Institute, Madison, Wisconsin.

Mikheev, V. P. 1964. Mortality rate of *Dreissena polymorpha* in anaerobic conditions. *In: Biology and Control of* Dreissena, B. K. Shegman, ed. pp. 65-68. Trudy Instituta Biologii Unutrennikhvod, 7(10), Academy of Sciences of the U. S. S. R., Institute of Biology of Inland Waters, Moscow, U. S. S. R.

Morton, B. 1969. Studies on the biology of *Dreissena polymorpha* (Pall.) I. General anatomy and morphology. *Proceedings of the Malacological Society of London* 38:301-321.

Nichols, J. S. 1993. Maintenance of the zebra mussel *(Dreissena polymorpha)* under laboratory conditions. *In: Zebra Mussels: Biology, Impact, and Control,* T. F. Nalepa and D. W. Schloesser, eds. pp. 733-749. Lewis Publishers, Boca Raton, Florida.

Paul, J. D. 1980. Upper temperature tolerance and the effects of temperature on byssus attachment in the queen scallop, *Chlamys opercularis* (L). *Journal of Experimental Marine Biology* 46:41-50.

Price, H. A. 1980. Seasonal variation in the strength of byssal attachment of the common mussel *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom* 60:1035-1037.

Price, H. A. 1982. An analysis of factors determining seasonal variation in the byssal attachment strength of *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom* 62:147-155.

Roberts, L. 1989. Zebra mussel invasion threatens U. S. waters. *Science* 249:1370-1372.

Stern, S. and Y. Achituv. 1978. Effects of temperature and salinity on the metabolism and byssal formation of *Brachidontes variabilis,* Krauss (Bivalvia). *Journal of Comparative Biochemistry and Physiology* 59A:101-105.

van Winkle, W., Jr. 1970. Effect of environmental factors on byssal thread formation. *Marine Biology* 7:143-148.

Yonge, C. M. 1962. On the primitive significance of the byssus in the Bivalvia and its effect in evolution. *Journal of the Marine Biological Association of the United Kingdom* 42:113-125.

Young, G. A. 1985. Byssus-thread formation by the mussel *Mytilus edulis:* effects of environmental factors. *Marine Ecology - Progress Series* 24:261-271.

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Changes in mussel (Bivalvia: Unionidae) fauna within the Kentucky portion of Lake Barkley since impoundment of the lower Cumberland River

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Abstract: Freshwater mussels (Bivalvia: Unionidae) were sampled at 74 sites in the Kentucky portion of Lake Barkley on the lower Cumberland River in 1994. Specimens were collected by SCUBA diving. Twenty 1 m^2 quadrats were sampled at each site. Four distinct habitats were sampled: submerged old river levees, overbanks or inundated floodplains, shorelines, and coves or embayments. Nine species were found: *Amblema plicata* (Say, 1817), *Anodonta suborbiculata* Say, 1831, *Fusconaia flava* (Rafinesque, 1820), *Megalonaias nervosa* (Rafinesque, 1820), *Obliquaria reflexa* Rafinesque, 1820, *Pyganodon grandis* (Say, 1829), *Quadrula nodulata* (Rafinesque, 1820), *Q. quadrula* (Rafinesque, 1820), and *Utterbackia imbecillis* (Say, 1829). A 1911 survey in the same Kentucky section of the river reported 25 species. Five species were found in 1994 that were not reported in 1911: *Anodonta suborbiculata, P. grandis, Q. nodulata, Q. quadrula,* and *U. imbecillis.* The other four species found in our survey, *Amblema plicata, F. flava, M. nervosa,* and *O. reflexa,* are the only remaining species that were present in 1911. This represents an 84% decline in original species and a 64% decline in species richness since 1911. Downstream from Barkley Dam the river remains in its original channel, and the bottom substratum is similar to the pre-impoundment riverbed. A 1981 survey of the tailwater reach from the dam to the Ohio River reported 21 species. Fifteen of these species were present in 1911 and six had not been reported previously. Seven of the species found in the lake in 1994 were also in the tailwater in 1981 while two species, *U. imbecillis* and *A. suborbiculata,* had not been reported. Impoundment of the Cumberland River after 1911 probably is responsible for much of the decline in the number of species and the change in species composition of this fauna. Pollution, commercial shell harvest, and invasion by non-native species could have contributed to the changes. Two byssal plaques of *Dreissena polymorpha* (Pallas, 1771) were observed on two unionid shells, but no live zebra mussels were found.

Key words: mussels, Unionidae, Cumberland River, Lake Barkley, impoundment

The Cumberland River headwaters are in the Cumberland Mountains of southeastern Kentucky. The river flows from eastern Kentucky through north-central Tennessee and western Kentucky to the Ohio River. The confluence with the Ohio River is at Ohio River mile (ORM) 923.0 at Smithland, Kentucky. Between 1916 and 1923, five locks and dams were constructed by the U. S. Army Corps of Engineers on the Cumberland River in the reach now in Lake Barkley (Johnson, 1978). These dams were designated Lock and Dam B through F. Only Dams E and F were located in Kentucky: Dam E at Cumberland River Mile (CRM) 66.3, completed in 1922, and Dam F at Eddyville, Kentucky, (CRM 43.6) completed in 1923. With the completion of Lock and Dam 52 in 1926 on the Ohio River (ORM 938.9) near Paducah, Kentucky, a minimum channel depth of 1.83 m could be maintained for navigation upstream and all of the shoals were inundated. When Barkley Dam was completed in 1965 at CRM 30.6, it replaced Dams B through F and formed Lake Barkley. Construction of Barkley Dam began on 1 July 1964, and the minimum pool elevation (107.9 m above mean sea level), inundating the original river channel and floodplains, was reached 16 February 1966 (Lowery *et al.*, 1990). Lake Barkley extends from Barkley Dam to Cheatham Dam in Dickson County, Tennessee, at CRM 148.7, a distance of approximately 190 km. The Kentucky-Tennessee border is at CRM 74.6 which was the upstream limit of our project.

The Cumberland River once supported one of the richest assemblages of freshwater mussels in the world. Historically at least 85 species have been reported (Starnes and Bogan, 1988). Before construction of dams on the lower Cumberland River, Wilson and Clark (1914) reported 47 species of mussels in a 1911 survey in the reach of the river from CRM 36.5 to 145 which is now under Lake Barkley. They reported 25 species within the Kentucky reach from CRM 36.5 to 73.0. The difference in number of

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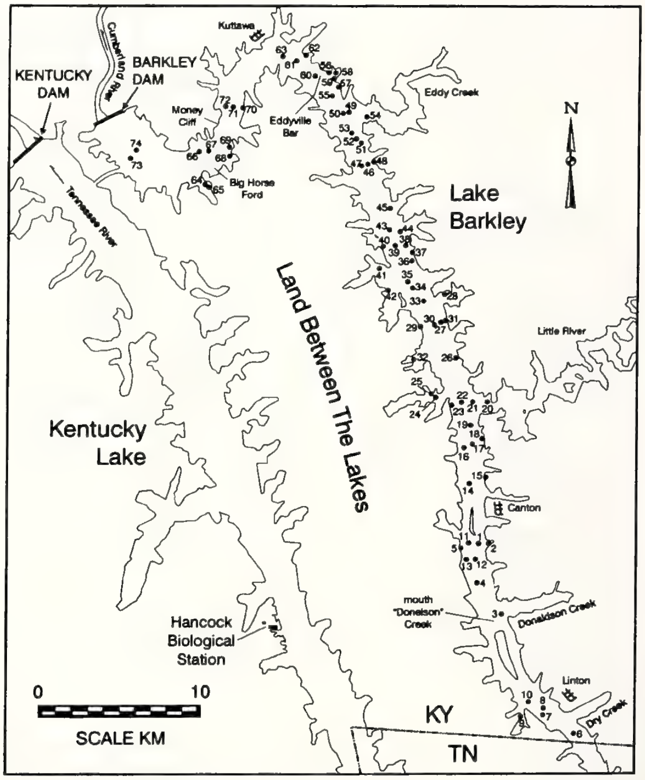
species can be explained in part perhaps by the difference in the extent of sampling within the two sections. Wilson and Clark sampled seven sites in Kentucky and 19 in the Tennessee section from approximately CRM 80 to 145. They examined approximately 1080 specimens from Kentucky and 2613 from the Tennessee section. More important than sample size and area, however, is the change in the character of the river which occurs in the vicinity of Clarksville, Tennessee (CRM 126). Here the Cumberland River leaves the Highland Rim province (Starnes and Etnier, 1986), and the gradient decreases which affects the mussel habitats. Ortmann (1924) pointed out the difference when he described the region upstream from Clarksville as belonging to what he defined as the Cumberlandian Region, which includes the middle and upper Cumberland and Tennessee Rivers. Historically, the Cumberlandian Region contained a rich mussel fauna. Downstream from Clarksville there were no Cumberlandian species, at least in the historical records. Archaeological data suggest that several Cumberlandian species once occurred as far downstream as CRM 26 (Casey, 1987) during or shortly after the dry Hypsithermal (8,700-5,000 YBP) (Franklin, 1994) when the Cumberland River would have been shallower and smaller. In general, as rivers increase in size, habitats become more uniform and the number of mussel species decreases. The present study compares the results of our 1994 mussel survey of the impounded area of Lake Barkley within Kentucky with that of Wilson and Clark (1914) prior to impoundment and Sickel (1982) for the Barkley Dam tailwater.

METHODS

The Kentucky portion of Lake Barkley from Barkley Dam at CRM 30.6 to the Kentucky-Tennessee Border (CRM 74.6) (a distance of 70.8 km) was examined between 26 July and 17 August 1994. Seventy-four sites were chosen to represent four basic habitats. The habitat types were the inundated old river levees, overbanks or submerged old floodplains, shorelines, and coves or embayments. These habitats were described by Sickel and Chandler (1982) for Kentucky Lake. Fig. 1 shows the locations of sample sites in Lake Barkley. The main channel with a depth ranging from 8-30 m was not sampled because preliminary surveys using mussel brails had located no mussels. Anaerobic conditions in the channel during summertime and deep, soft, organic silt have eliminated most of the unionid mussels from the channel in the Kentucky portion of the reservoir.

Mussels were collected quantitatively by SCUBA divers using 1 m^2 quadrats. A quadrat frame consisted of welded 1.27 x 0.64 cm aluminum bar stock. The 1 m^2 frame was subdivided into quarters by cross bars to provide smaller areas for the divers to search. Because most of the collecting occurred with no visibility, the divers had to feel the frame for orientation. With the frame divided into quarters, the divers were able to search the entire m^2 area without omitting part or unnecessarily repeating sections of the quadrat. Twenty quadrats were sampled at each site. All mussels collected within each quadrat were brought onto the boat for identification, measurement, weighing, and determination of reproductive condition. All that is reported here is the species composition and percentage abundance. Other data will be reported in a final report to the Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky.

Sites surveyed by Wilson and Clark (1914) located within the Kentucky portion of Barkley Lake are indicated in Fig. 1. Current navigation charts show the landmarks designated by Wilson and Clark (1914). These sites and their approximate river miles are Big Horse Ford (CRM 36.5), Money Cliff (CRM 39), Kuttawa (CRM 41), Eddyville Bar (CRM 44), Canton (CRM 62), mouth of "Donelson" [Donaldson] Creek (CRM 67), and Linton


Kuttawa
KENTUCKY DAM
Cumberland River
BARKLEY DAM
Money Cliff
Eddyville Bar
Eddy Creek
N
Big Horse Ford
Tennessee River
Lake Barkley
Land Between The Lakes
Little River
Kentucky Lake
Canton
mouth "Donelson" Creek
Donaldson Creek
Hancock Biological Station
Linton
Dry Creek
0
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SCALE KM
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TN
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Fig. 1. Location of sample sites (numbers 1-74) in the 1994 mussel survey in Lake Barkley and the seven locations indicated in the Wilson and Clark (1914) survey of the lower Cumberland River in Kentucky (Big Horse Ford, Money Cliff, Kuttawa, Eddyville Bar, Canton, mouth of 'Donelson' Creek, and Linton).

(CRM 73). Sites reported by Sickel (1982) were located downstream from Barkley Dam between CRM 4.6 and 30.3.

Because zebra mussels, *Dreissena polymorpha* (Pallas, 1771), have been found in previous years in Lake Barkley, we examined each unionid shell carefully for the presence of zebra mussels or their byssal plaques.

RESULTS

Table 1 lists all mussel species reported by Wilson and Clark (1914), Sickel (1982), and the present study from the Kentucky portion of the lower Cumberland River including Lake Barkley and the tailwater reach of Barkley Dam. Nomenclature agrees with that of Turgeon *et al.* (1988) except where noted otherwise in the table. The number of specimens examined in 1994 was 473. Nine species were collected: *Amblema plicata* (Say, 1817), *Pyganodon grandis* (Say, 1829), *Utterbackia imbecillis* (Say, 1829), *Anodonta suborbiculata* Say, 1831, *Fusconaia flava* (Rafinesque, 1820), *Megalonaias nervosa* (Rafinesque, 1820), *Obliquaria reflexa* Rafinesque, 1820, *Quadrula nodulata* (Rafinesque, 1820), and *Q. quadrula* (Rafinesque, 1820). The percentage abundance of each species is given in Table 1 along with values calculated from data in Wilson and Clark (1914) and Sickel (1982).

Wilson and Clark (1914) collected 25 species of unionid mussels from the Kentucky portion of the lower Cumberland River between CRM 36.5 and 73. Sickel (1982) listed 21 species surviving in the tailwater of Barkley Dam (CRM 2.5-30.6). Nine species not found alive by Sickel (1982) but reported by Wilson and Clark (1914) were *Actinonaias ligamentina* (Lamarck, 1819), *Cyclonaias tuberculata* (Rafinesque, 1820), *Lampsilis abrupta* (Say, 1831), *L. ovata* (Say, 1817), *L. teres* (Rafinesque, 1820), *Obovaria subrotunda* (Rafinesque, 1820), *O. retusa* (Lamarck, 1819), *Plethobasus cooperianus* (Lea, 1834), and *Quadrula fragosa* (Conrad, 1835). Six species found by Sickel (1982) but not reported by Wilson and Clark (1914) were *Pyganodon grandis, Arcidens confragosus* (Say, 1829), *Lasmigona complanata* (Barnes, 1823), *Q. nodulata, Q. quadrula,* and *Truncilla donaciformis* (Lea, 1828). *L. complanata* and *T. donaciformis* were reported by Wilson and Clark (1914) in other areas of the Cumberland River and may have been present but overlooked in the lower region. The Barkley Dam tailwater experienced a 36% decline in original species since 1911. However, with the addition of the six species that were not reported by Wilson and Clark (1914), the overall decline in species richness is only 16% in the tailwater.

Comparison between the pre-impoundment lower Cumberland River (Wilson and Clark, 1914) and impounded Lake Barkley reveals an 84% decline in original species. Twenty-one of the original 25 species reported by Wilson and Clark (1914) have been eliminated from the Kentucky portion of the Cumberland River now within Lake Barkley. The only four species still surviving in the Kentucky portion of Lake Barkley that were present in 1911 are *Amblema plicata, Fusconaia flava, Megalonaias nervosa,* and *Obliquaria reflexa*. Five species have invaded since impoundment: *Pyganodon grandis, Utterbackia imbecillis, Anodonta suborbiculata, Quadrula nodulata,* and *Q. quadrula*. With the addition of these five species, the species richness declined only 64% between 1911 to 1994. Comparisons between the tailwater and the reservoir reveal that seven species are common to both areas. Two species were not found in Barkley Dam tailwater that occur in the lake: *U. imbecillis* and *A. suborbiculata*. The impoundment is lacking 67% of the current tailwater species. Those absent from the impoundment are *Arcidens confragosus, Ellipsaria lineolata* (Rafinesque, 1820), *Elliptio crassidens* (Lamarck, 1819), *Elliptio dilatata* (Rafinesque, 1820), *Fusconaia ebena* (Lea, 1831), *Lasmigona complanata, Leptodea fragilis* (Rafinesque, 1820), *Ligumia recta* (Lamarck, 1819), *Pleurobema cordatum* (Rafinesque, 1820), *Potamilus alatus* (Say, 1817), *Quadrula metanevra* (Rafinesque, 1820), *Q. pustulosa* (Lea, 1831), *Tritogonia verrucosa* (Rafinesque, 1820), and *Truncilla donaciformis*.

Although 25 species were present in the Kentucky portion of the lower Cumberland River in 1911, 88% of the specimens reported by Wilson and Clark (1914) belonged to only four species: *Elliptio crassidens* (3.1%), *Fusconaia ebena* (31.9%), *Megalonaias nervosa* (12.3%), and *Pleurobema cordatum* (40.7%). In the 1981 tailwater survey (Sickel, 1982; Sickel and Chandler, 1996), the species distribution was more even. Sampling bias toward the larger commercial species may have influenced the results of Wilson and Clark (1914). They used mussel brails and examined the harvest of commercial musselers for much of their data. Sickel (1982) used a combination of brail and SCUBA for sampling, and this may have resulted in a more complete representation of the actual distribution of the mussel species. Regardless of the differences in collecting methods, the same four species were most abundant in the Barkley Dam tailwater in 1981 as in the unimpounded river in 1911. Those four species constituted 60% of the fauna in 1981: *Elliptio crassidens* (10.1%), *Fusconaia ebena* (13.5%), *Megalonaias nervosa* (29.9%), and *Pleurobema cordatum* (7.2%). Two other species, *Quadrula quadrula* and *Amblema plicata*, also had a 7.2% abundance. These data show a marked decline in percent abundance of the two most abundant commercial species, *F. ebena* and *P. cordatum*, from 1911 to 1981 in the still riverine section of the river, and their complete disappearance from the Kentucky portion of Lake Barkley.

Table 1. Mussel species reported alive and their percentage abundance in the Kentucky portion of the lower Cumberland River including the region inundated by Lake Barkley.

Taxa[1]	Wilson and Clark (1911)[2]	Sickel (1981)[3]	Present Study (1994)
Actinonaias ligamentina (Lamarck, 1819)	0.37	—	—
Amblema plicata (Say, 1817)	0.65	7.21	17.76
Anodonta suborbiculata Say, 1831	—	—	1.90
Arcidens confragosus (Say, 1829)	—	0.90	—
Cyclonaias tuberculata (Rafinesque, 1820)	0.46	—	—
Ellipsaria lineolata (Rafinesque, 1820)	1.02	3.24	—
Elliptio crassidens (Lamarck, 1819)	3.12	10.09	—
E. dilatata (Rafinesque, 1820)	0.37	0.18	—
Fusconaia ebena (Lea, 1831)	31.94	13.15	—
F. flava (Rafinesque, 1820)	0.65	1.80	0.21
Lampsilis abrupta (Say, 1831)	0.28	—	—
L. ovata (Say, 1817)	0.37	—	—
L. teres anodontoides (Lea, 1931)[4]	0.09	—	—
L. teres teres (Rafinesque, 1820)	0.56	—	—
L. complanata (Barnes, 1823)	—	1.08	—
Leptodea fragilis (Rafinesque, 1820)	0.37	1.08	—
Ligumia recta (Lamarck, 1819)	0.56	0.54	—
Megalonaias nervosa (Rafinesque, 1820)	12.31	29.91	2.53
Obliquaria reflexa Rafinesque, 1820	0.46	4.68	3.80
Obovaria retusa (Lamarck, 1819)	0.19	—	—
O. subrotunda (Rafinesque, 1820)	0.46	—	—
Plethobasus cooperianus (Lea, 1834)	0.65	—	—
Pleurobema cordatum (Rafinesque, 1820)	40.74	7.21	—
Potamilus alatus (Say, 1817)	0.74	3.78	—
Pyganodon grandis (Say, 1829)[5]	—	0.18	2.32
Quadrula fragosa (Conrad, 1835)	1.57	—	—
Q. metanevra (Rafinesque, 1820)	1.67	2.52	—
Q. nodulata (Rafinesque, 1820)	—	1.44	6.55
Q. pustulosa (Lea, 1831)	0.73	0.72	—
Q. quadrula (Rafinesque, 1820)	—	7.21	64.67
Tritogonia verrucosa (Rafinesque, 1820)	0.28	2.88	—
Truncilla donaciformis (Lea, 1828)	—	0.18	—
Utterbackia imbecillis (Say, 1829)[5]	—	—	0.21
Total Species	25	21	9
Total Number Collected	1080	555	473

[1] Scientific names currently recognized by Turgeon *et al.* (1988) except where noted.
[2] Calculated from data of Wilson and Clark (1914).
[3] Calculated from Barkley Dam tailwater survey of Sickel (1982).
[4] Nomenclature of Ortmann and Walker (1922).
[5] Nomenclature of Hoeh (1990).

Within the reservoir in 1994, 65% of the collected specimens belonged to a single species, *Quadrula quadrula.* This species only had a 7% abundance in the tailwater in 1981, and it was not reported in 1911. Wilson and Clark (1914) reported *Q. fragosa* but not *Q. quadrula*. Today, *Q. fragosa* does not occur in Lake Barkley or the tailwater area, and it has been placed on the Federal Endangered Species List (USFWS, 1995). Perhaps it was displaced by *Q. quadrula*, just as at present we are observing *Q. quadrula* being displaced by *Q. apiculata* (Say, 1829) in parts of Kentucky Lake of the Tennessee River.

The second most abundant species in Lake Barkley was *Amblema plicata* (17.8%). *Quadrula quadrula* and *A. plicata* constituted 82.4% of the mussel fauna in the Kentucky portion of the reservoir. They are large-river, Interior Basin forms that have adapted well to the silty substratum of the reservoir.

Quadrula nodulata and *Anodonta suborbiculata* are recent introductions into the Cumberland River. Johnson (1980) stated that both originated in the Mississippian

Region and invaded the Ohio River after the Pleistocene. *Q. nodulata* was observed in the Cumberland River for the first time in 1981 (Sickel, 1982; Sickel and Chandler, 1996) while *A. suborbiculata* was first observed in 1982 (Sickel, 1983).

Byssal plaques left by zebra mussels, *Dreissena polymorpha,* were found on two unionid shells. No live zebra mussels were found in this survey of Lake Barkley in 1994.

DISCUSSION

There are several explanations for the decline in species richness caused by impoundments. Before impoundment the lower Cumberland River was relatively shallow with a bottom consisting of gravel with patches of sand and silt much like the present tailwater section downstream from Barkley Dam. In the tailwater, depths during low discharge periods are maintained at a minimum of 3-4 m by Dam 52 on the Ohio River. This provides some flow regulation, but conditions probably are similar to the river before impoundment. Today much of the bottom consists of large gravel, sandy gravel, and some sandy mud behind bars or along steep banks. Within several kilometers of the Ohio River the bottom becomes more silty as the water is backed up by the Ohio River. Barkley Dam raises the water level in the reservoir by 15.8-17.4 m. This creates a deep channel that accumulates a fine, highly organic sediment especially during periods of low discharge. During these periods the deeper water becomes stagnant and anaerobic. Silt range data from the Nashville District Army Corps of Engineers (pers. comm.) indicates 1-3 m of silt accumulated from 1965 to 1984 in many areas of the main channel. Mussel habitat that was present in the channel in 1965 when the dam was closed has been covered by silt. Under anaerobic conditions, it is unlikely that the mussels could have extricated themselves from the accumulating silt.

Other changes in the riverine habitat after impoundment can result in loss of fish hosts for the larval stage (glochidium) of the mussel. Fuller (1974) suggested that even if the fish hosts are available, the water quality and habitat conditions in the impounded area might not be suitable for juvenile mussels to survive. Most of the sites examined in 1994 had at least 0.5-1.0 cm of silt over various substrata from hard clay to sand and gravel. In depressions, coves, and deeper floodplain or overbank sites, the silt was deeper than a diver's arm which could be inserted easily up to the shoulder. Ellis (1936) reported that freshwater mussels cannot survive if a layer of silt deposited over them is greater than 0.64 cm deep due to interference with feeding behavior. The majority of mussel species survive in areas where rapid siltation does not occur, preferring areas with swift current (Coker *et al.*, 1921). This could explain the loss of 84% of the unionid species in Lake Barkley reservoir since impoundment of the Cumberland River. Other researchers (Bates, 1962; Fuller, 1974; Miller *et al.*, 1984; Williams *et al.*, 1992) have reported significant declines in unionid species richness after impoundment of rivers. Other factors that could contribute to the decline in species richness include pollution, commercial shell harvest, erosion caused by barge traffic, and invasion of non-native species.

Van der Schalie (1939) recognized that large rivers such as the lower Tennessee River support fewer species than their middle and upper reaches. The diverse Cumberlandian fauna of the Tennessee River extends only as far downstream as the edge of the Fall Line (van der Schalie, 1939) where the last major shoals occurred prior to impoundment. Today, impoundments have flooded these areas and eliminated many of the original species (Stansbery, 1964) just as they have in the Cumberland River. Williams *et al.* (1992) reported a loss of 37% of the species in the impounded segments of the Black Warrior and Tombigbee Rivers. Today the tailwaters of the large dams could provide the only suitable habitat for many of the riverine species.

The Barkley Dam tailwater survey (Sickel, 1982; Sickel and Chandler, 1996) demonstrates that 60% (15 of 25) of the original unionid species that were present in 1911 remain today downstream from Barkley Dam. However, 67% of the present tailwater species were not found in the reservoir. New species have invaded both the tailwater and the reservoir. These species are more tolerant to silt and probably utilize fish hosts that are common. Houp (1993) stated that mussel communities in silty habitats will consist of species with many host fishes and less specific habitat requirements.

Future surveys in the Barkley Dam tailwater and Lake Barkley need to be conducted to determine the fate of the species that have survived impoundment. Most likely, the remaining species in Lake Barkley will survive and additional species will invade the impounded region from the Ohio River or southern rivers. The tailwater fauna might not fare as well. In the 1981 survey (Sickel, 1982; Sickel and Chandler, 1996), there was little evidence of recruitment for the less common species. Based on external shell annuli, many individuals were at least as old as Barkley Dam (16 years in 1981). Another survey is needed in the tailwater region to determine which species have survived and which are reproducing.

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LITERATURE CITED

Bates, J. M. 1962. The impact of impoundment on the mussel fauna of Kentucky Reservoir, Tennessee River. *The American Midland Naturalist* 68 (1):232-236.

Casey, J. L. 1987. Aboriginal and modern mussel assemblages of the lower Cumberland River. *Southeastern Archaeology* 6(2):115-124.

Coker, R. E., A. F. Shira, H. W. Clark, and A. D. Howard. 1921. Natural history and propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries* 37:77-181.

Ellis, M. M. 1936. Erosion silt as a factor in aquatic environments. *Ecology* 17(1):29-42.

Franklin, S. B. 1994. Late Pleistocene and Holocene vegetation history of Land Between the Lakes, Kentucky and Tennessee. *Transactions of the Kentucky Academy of Science* 55(1-2):6-19.

Fuller, S. L. H. 1974. Clams and mussels (Mollusca: Bivalvia). *In: Pollution Ecology of Freshwater Invertebrates*, C. W. Hart, Jr. and S. L. H. Fuller, eds. pp. 215-273. Academic Press, New York.

Hoeh, W. R. 1990. Phylogenetic relationships among eastern North American *Anodonta* (Bivalvia: Unionidae). *Malacological Review* 23:63-82.

Houp, R. E. 1993. Observations on long-term effects of sedimentation of freshwater mussels (Mollusca: Unionidae) in the North Fork of Red River, Kentucky. *Transactions of the Kentucky Academy of Science* 54(3-4):93-97.

Johnson, L. R. 1978. *Engineers on the Twin Rivers.* U. S. Army Engineer District, Nashville, Tennessee. 329 pp.

Johnson, R. I. 1980. Zoogeography of North American Unionacea (Mollusca: Bivalvia) north of the maximum Pleistocene glaciation. *Bulletin of the Museum of Comparative Zoology, Harvard University* 149(2):77-189.

Lowery, J. F., P. H. Counts, F. D. Edwards, and J. W. Garrett. 1990. *Water Resources Data —Tennessee, Water Year 1989.* Report No. USGS-WDR-TN-89-1. United States Geological Survey, Nashville, Tennessee. 282 pp.

Miller, A. C., L. Rhodes, and R. Tippit. 1984. Changes in the naiad fauna of the Cumberland River below Lake Cumberland in central Kentucky. *The Nautilus* 98(3):108-110.

Ortmann, A. E. 1924. The naiad-fauna of the Duck River in Tennessee. *American Midland Naturalist* 9:18-62.

Ortmann, A. E. and B. Walker. 1922. On the nomenclature of certain North American naiades. *Occasional Papers, Museum of Zoology, University of Michigan* 112:1-75.

Sickel, J. B. 1982. *A Survey of the Freshwater Mussels of the Lower Cumberland River from Barkley Dam Tailwater Downstream to the Ohio River.* Report for the Nashville District, United States Army Corps of Engineers, Nashville, Tennessee. 24 pp.

Sickel, J. B. 1983. *Phase II, Barkley Lake Survey, Cumberland River Miles 35 - 47.* Report for the Nashville District, United States Army Corps of Engineers, Nashville, Tennessee. 119 pp.

Sickel, J. B. and C. C. Chandler. 1982. *Commercial Mussel and Asiatic Clam Fishery Evaluation.* Report of Project No. 2-367-R, Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky. 77 pp.

Sickel, J. B. and C. C. Chandler. 1996. Unionid fauna of the lower Cumberland River from Barkley Dam to the Ohio River, Kentucky (Mollusca: Bivalvia: Unionidae). *Transactions of the Kentucky Academy of Science* 57(1):33-46.

Stansbery, D. H. 1964. The Mussel (Muscle) Shoals of the Tennessee River revisited. *Annual Report for 1964 of the American Malacological Union*, pp. 25-28.

Starnes, L. B. and A. E. Bogan. 1988. The mussels (Mollusca: Bivalvia: Unionidae) of Tennessee. *American Malacological Bulletin* 6(1):19-37.

Starnes, W. C. and D. A. Etnier. 1986. Drainage evolution and fish biogeography of the Tennessee and Cumberland Rivers drainage realm. *In: Zoogeography of North American Freshwater Fishes*, C. H. Hocutt and E. O. Wiley, eds. pp. 325-361. John Wiley and Sons, New York.

Turgeon, D. D., A. E. Bogan, E. V. Coan, W. K. Emerson, W. G. Lyons, W. L. Pratt, C. F. E. Roper, A. Scheltema, F. G. Thompson, and J. D. Williams. 1988. *Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks.* American Fisheries Society Special Publication 16, 277 pp.

United States Fish and Wildlife Service [USFWS]. 1995. *Endangered and Threatened Wildlife and Plants.* 50 CFR 17.11 and 17.12.

van der Schalie, H. 1939. Additional notes on the naiades (fresh-water mussels) of the lower Tennessee River. *American Midland Naturalist* 22(2):452-457.

Williams, J. D., S. L. H. Fuller, and R. Grace. 1992. Effects of impoundments on freshwater mussels (Mollusca: Bivalvia: Unionidae) in the main channel of the Black Warrior and Tombigbee Rivers in western Alabama. *Bulletin of the Alabama Museum of Natural History* 13:1-10.

Wilson, C. B. and H. W. Clark. 1914. *The Mussels of the Cumberland River and Its Tributaries.* United States Fish Commission, United States Bureau of Fisheries Document 781:1-63.

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Genetic differentiation in and management recommendations for the freshwater mussel, *Pyganodon grandis* (Say, 1829)

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Abstract: The giant floater, *Pyganodon grandis* (Say, 1829), was once abundant in lakes, rivers, and creeks in nearly all kinds of substrata from the east coast to the Rocky Mountains. Surveys of the giant floater revealed that the number of populations and population sizes have declined significantly at the western margin of the distribution. We surveyed five populations for variation of allozymes, male mtDNA, and female mtDNA to provide the data needed to guide conservation and restoration efforts in Colorado. Both allozyme and female mtDNA revealed significant variation between drainages, but far more variation was revealed between drainages in the male mtDNA. The degree of differentiation among drainages is sufficiently great that we recommend that new populations be founded with individuals taken from natural populations within the same drainage.

Key words: Unionidae, *Pyganodon*, population structure, management

Freshwater mussels of the families Unionidae and Margaritiferidae are worldwide in distribution but reach their greatest diversity in North America, with about 297 recognized taxa (Williams *et al.,* 1992). During the past 30 years, species diversity of native mussels and population sizes have declined dramatically throughout the United States and Canada. The primary reasons for the decline are habitat destruction, habitat alteration, and the introduction of non-endemic mollusks, especially the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) (Hunter and Bailey, 1991, 1992). According to Stolzenburg (1995), freshwater bivalves are now the second most endangered group of organisms in North America, with 57% of species either critically imperiled, imperiled, or rare. One out of ten North American mussel species could already be extinct.

Pyganodon grandis (Say, 1829) (formerly *Anodonta grandis grandis*; see Hoeh, 1990) is commonly known as the giant floater. The giant floater is broadly distributed throughout the Mississippi-Missouri river drainage, the St. Lawrence drainage, the Canadian Interior Basin from western Ontario to Alberta, and in the Gulf of Mexico drainage area of Louisiana and Texas (Burch, 1975). However, it has very limited occurrence at the western edge of its distribution on the Great Plains. According to Wu (1989), the giant floater is in critical condition in Colorado due to habitat destruction and pollution. Recently, governmental agencies and academic scientists are developing propagation and reintroduction programs for the giant floater. The goal is to restore the giant floater to its previous range. However, recent restocking programs, most notably the breeding and restocking of the dusky seaside sparrow in Florida (Avise and Nelson, 1989; Avise 1994), have clearly demonstrated that we need to examine genetic variability and use the genetic data to design breeding and restocking programs. Should all mussels in Colorado be considered as members of a single homogeneous population? Or are mussels from different drainages substantially differentiated? We need to answer these questions with studies of genetic variation and population structure before we can make informed decisions regarding the reintroduction and management of the giant floater.

Management of the giant floater is made more complex by its unusual life cycle. The zygotes are brooded in a female marsupium (modified gill lamellae), where they develop through the veliger stage. However, the veliger, called a glochidium in the family Unionidae, has evolved into a highly modified parasitic larva. Because glochidia are obligate parasites on freshwater fishes, the host fish species are necessary for the recruitment and dispersal of mussels. When considering the reintroduction and management of the mussels, it is necessary to survey the local icthyofauna for suitable hosts. Fortunately, more than 30 fish species can be utilized by the giant floater as host species (Fuller, 1974; Trdan and Hoeh, 1982).

Until recently the inheritance of mtDNA in animals was thought to be strictly maternal. But recently an unusual pattern of mtDNA inheritance, *i. e.* double uniparental inheritance, has been reported in the blue mussel, *Mytilus edulis* Linné, 1758 (Skibinski *et al.*, 1994; Zouros *et al.*, 1994), and in the giant floater, *Pyganodon grandis* (Liu *et al.*, 1996). These species of bivalves have, in addition to the normal maternally inherited mtDNA system, a second, paternally inherited mtDNA system. Studies of mtDNA have been particularly useful for describing population structure, partly due to their uniparental inheritance and lack of recombination, and partly because patterns of geographic variation revealed by mtDNA can differ dramatically from patterns revealed by allozyme variation (Karl and Avise, 1992; reviewed in Mitton, 1994). Therefore, we estimated population structure of the giant floater with protein electrophoresis and paternal and maternal mitochondrial DNA restriction fragment length polymorphisms (mtDNA RFLPs). After analyzing the genetic data, we formulated recommendations for the management and restoration of the giant floater in Colorado.

The objectives of this study were to survey the current mussel distribution and to describe genetic variation within and among populations of the giant floater in Colorado.

MATERIALS AND METHODS

Giant floaters are usually partially buried in the substratum with the posterior portion protruding. We searched for mussels by feeling with hands and feet along the surface of the substratum while wading, snorkeling, and/or scuba diving.

DRAINAGE SURVEYED

The rivers in Colorado can be grouped into ten drainages (Brandauer and Wu, 1978; Fig. 1). Historically, the giant floater was only reported from three: the Arkansas River, the Republican River, and the South Platte River drainages. Therefore, only these three drainages were surveyed to determine the current distribution of the mussel. All of the known historical site reports for Colorado have been compiled from the literature and are listed in Table 1. We revisited all historical sites except Washington Park (Fig. 1).

MITOCHONDRIAL DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Mussel populations from three drainage systems were sampled for the survey of genetic variation: (1) Arkansas River drainage: Colorado Fuel and Iron Reservoir No. 2 (T21S, R65W, s34/35, Pueblo Co., Colorado), 15 specimens; (2) Republican River drainage: Flagler Reservoir (T9S, R50W, s4/5/9/10, Kit Carson Co., Colorado), 16 specimens; (3) South Platte River drainage: slough near Lake McConaughy (T14N, R38W, s4, Keith Co., Nebraska), 8 specimens. Genetic data for two more mussel populations, the Cherry Creek Reservoir population in the South Platte River drainage and the Pueblo Reservoir population in the Arkansas River drainage, were available from Liu *et al.* (1996). We assessed gender based on the presence or absence of the marsupial gill and verified by the histology. Mussels with a marsupium are female, and mussels lacking a marsupium are male. In our samples, all individuals with a marsupium also carried glochidia. Total DNA was extracted from gonadal tissue for each individual. Five restriction enzymes, *Ase*I, *Eco*RI, *Hae*III, *Hind*III, and *Hin*fI, were used to digest the total DNA except that the McConaughy samples were only digested with *Eco*RI. Mitochondrial DNA fragments were detected and scored as described in Liu *et al.* (1996). Mitochondrial DNA data were analyzed by the RESTSITE computer program (Miller, 1991).

ALLOZYME ELECTROPHORESIS

For each individual, 0.1-0.2 g of gonadal tissue was homogenized with buffer (0.01M sodium phosphate, 0.001M EDTA, 0.001M mercaptoethanol, pH 7.0). The homogenates were electrophoresed, stained, and scored as described in Liu *et al.* (1996). Eight enzyme systems were surveyed and 12 loci were resolved. Allozyme data were analyzed by the BIOSYS computer program (Swofford and Selander, 1981).

RESULTS

DRAINAGES SURVEYED

The surveys of the Arkansas River drainage, the Republican River drainage, and the South Platte River drainage beyond those already cited in the literature (Table 1) did not reveal any new populations of mussels.

Arkansas River Drainage

Pueblo Reservoir. This population was founded from the population in the Colorado Fuel and Iron Reservoir No. 3. In 1983, the water level was lowered in Colorado Fuel and Iron Reservoir No. 3 for scheduled outlet valve replacement and maintenance. When thousands of *Pyganodon grandis* became exposed, Herrmann and Fajt (1985) transferred 412 adult mussels from the Colorado Fuel and Iron Reservoir No. 3 to the newly formed Pueblo Reservoir. Twelve specimens were collected in September 1993 from the north marina area using chest waders and a long-handled dip net. Five were male and seven were

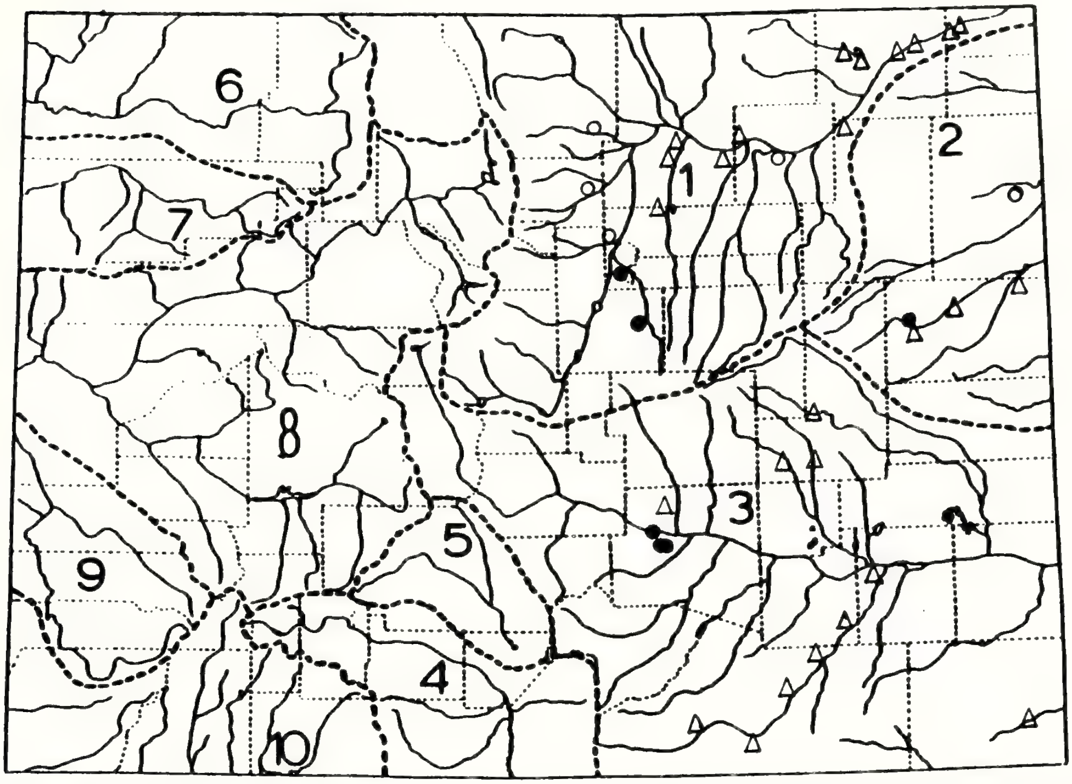

Fig. 1. Drainage systems in Colorado (modified from Wu, 1989): (1) South Platte drainage; (2) Republican drainage; (3) Arkansas drainage; (4) Rio Grande drainage; (5) San Luis Valley drainage; (6) Yampa drainage; (7) White drainage; (8) Colorado drainage; (9) Dolores drainage; (10) San Juan drainage. The light dotted lines (........) mark county boundaries; heavy lines (-------) indicate drainage areas. Symbols indicate historical sites of the freshwater mussel, *Pyganodon grandis*, in Colorado: (●) mussel population present during 1993-1994 survey; (O) no mussels found during 1993-1994 survey; (Δ) additional sites surveyed during 1993-1994 survey, no mussels found.

female. This population is persisting despite the fluctuations in water levels from year to year.

Colorado Fuel and Iron Reservoir No. 2. Forty-five live specimens were found and collected from the northwestern side of the reservoir during a one-hour search in June 1994. Live specimens were collected from a depth of 0.3-1.2 m on coarse sand to clay substratum. Mantle and gill tissues of all live specimens were infected with water mites (*Unionicola* sp.). The ratio of males to females was approximately 2:1.

Colorado Fuel and Iron Reservoir No. 3. Pyganodon grandis was present in this reservoir that is contiguous with Reservoir No. 2. The outlet of Reservoir No. 3 flows directly by gravity into Reservoir No. 2. Reservoir No. 2 and No. 3 were both constructed in 1903.

Republican River Drainage

Flagler Reservoir. In June 1994, 38 live specimens were found and collected at the west side of the reservoir from a depth of 1.8-4.5 m on clay substratum. This was the only population in Colorado not infected with water mites. The ratio of males to females was approximately 3:1.

Black Wolf Creek. In 1985, Herrmann and Fajt visited the vicinity of Black Wolf Creek site. They found four shell fragments after searching about a mile segment along the creek. We revisited this site in 1994. After searching along the creek for an hour, no specimens were found. In agreement with Herrmann and Fajt (1985), we concluded that the population in Black Wolf Creek has become extinct.

South Platte River Drainage

Cherry Creek Reservoir. Six live specimens were found and collected at the south side of the reservoir during a half-hour search in October 1993. Another 15 specimens were found and collected in the same area during a half-hour search in November 1993. All live specimens were collected from a depth of 0.3-1.2 m. The sediments contained various mixtures of particles from clay to coarse sand. Mantle and gill tissues of all live specimens were infested with water mites (*Unionicola* sp.). The ratio of males to females was approximately 1:1.

Mayham Lake. A two-hour search yielded not even a single fragment of a shell. At this time, it appears this

Table 1. Historical sites of the freshwater mussel, *Pyganodon grandis*, in Colorado.

Population name	Year	County	Coordinates	Locality	River drainage	Notes
Black Wolf Creek	1889	Yuma	unknown	pool in Black Wolf Creek, 1.5 mi N and 1 mi W of Beecher Island	Republican	revisited in July 1994, no mussels found
Boulder	1912	Boulder	unknown	30 mi N of Denver	South Platte	surveyed Boulder area in October 1993 and July 1994, no mussels found
Boyd Lake Reservoir	1978	Larimer	T6N, R68W, s32	Boyd Lake Recreation Area	South Platte	revisited in October and November 1993, found 2 live mussels
Mayham Lake	1985	Adams	T3S, R68W, s6	1 mi S of Westminster	South Platte	revisited in October 1993, no mussels Found
Flagler Reservoir	1985	Kit Carson	T9S, R50W, s4/5/9/10	34 mi E of Limon	Republican	revisited in June 1994, mussel population present
Pueblo Reservoir	1985	Pueblo	T20S, R66W, s33/36	10 mi W of Pueblo	Arkansas	revisited in September 1993, mussel population present
Colorado Fuel and Iron Reservoir #2	1985	Pueblo	T21S, R65W, s34/35	9 mi S of Pueblo	Arkansas	revisited in June 1994, mussel population present
Colorado Fuel and Iron Reservoir #3	1985	Pueblo	T21S, R65W, s 33/34	9 mi S of Pueblo	Arkansas	revisited in June 1994, mussel population present
Washington Park	1986	Denver	T4S, R68W, s14	Denver	South Platte	not surveyed
Cherry Creek Reservoir	1986	Arapahoe	T5S, R68W, s 3/11	7 mi SE of Denver	South Platte	revisited in October and November 1993, mussel population present
Morgan	1989	Morgan	unknown	Ft. Morgan	South Platte	surveyed Ft. Morgan area in July 1994, no mussels found

population has probably become extinct. In 1983, Herrmann and Fajt (1985) found many giant floaters representing nearly all age classes. Complete drainage of the lake in late 1983 and 1984 probably killed all mussels.

Boyd Lake Reservoir. In October 1993, a 90-min search by wading, snorkeling, and scuba diving yielded just one live individual, but hundreds of large empty shells were found. Another live specimen was found during a similar search in November 1993. Both live individuals were heavily infected with water mites (*Unionicola* sp.). The presence of water mites in the mussels either reflects poor health of the population or affects the health of the population. In either case, this population is on the verge of extinction.

Without more specific site information, it is impossible to locate the exact Ft. Morgan site. Thus, we surveyed Jackson Reservoir and Empire Reservoir near Ft. Morgan. Both reservoirs had suitable substratum but no mussels were found. Both reservoirs are drained periodically for maintenance; if the mussels ever existed in the reservoir, this fluctuation in the water level probably killed the mussels.

Without more specific site information, it is impossible to locate the exact Boulder site. We surveyed Boulder Creek and Fourmile Canyon, but we found no mussels.

Slough near Lake McConaughy. Thirty-five live specimens were found during an hour search. Eight specimens were collected, three females and five males. All live specimens were collected from a depth of 0.9-1.1 m. The sediments contained predominantly mud.

MITOCHONDRIAL DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Males have different mtDNA haplotypes than females. Within each of the populations, the difference between males and females ranges from 6.07-8.90% (Table 2). Maternal mtDNA haplotypes were very similar among localities, regardless of whether they were collected from Pueblo Reservoir, Colorado Fuel and Iron Reservoir No. 2, Flagler Reservoir, Cherry Creek Reservoir, or the slough near Lake McConaughy. The divergence is estimated to range from 0-0.5% (Table 2). The paternal mitochondrial genotypes were similar in Cherry Creek Reservoir and the slough at Lake McConaughy, but the divergence between these and the other localities was 11% (Table 2).

ALLOZYME ELECTROPHORESIS

Eight enzyme systems were examined and 12 loci were resolved. Of these 12 loci, three are polymorphic: phosphoglucomutase-1, phosphoglucomutase-2, and isoci-

Table 2. Estimated sequence divergence (Nei, 1978) in *Pyganodon grandis*. The diagonal line presents the divergence within populations between male and female mtDNA. The triangle of data above the diagonal presents the divergence among populations estimated with paternal mtDNA. The triangle of data below the diagonal presents the divergence among populations estimated with maternal mtDNA.

	Cherry Creek	Colorado Fuel and Iron	Flagler	McConaughy	Pueblo
Cherry Creek	6.41 ± 3.0%	11.61 ± 11.0%	11.46 ± 11.0%	0.00 ± 0.0%	11.28 ± 11.0%
Colorado Fuel and Iron	0.46 ± 0.4%	6.15 ± 3.2%	0.06 ± 0.0%	_[2]	0.03 ± 0.0%
Flagler	0.50 ± 0.5%	0.00 ± 0.0%	6.07 ± 3.3%	_[2]	0.00 ± 0.0%
McConaughy	0.00 ± 0.0%[1]	0.00 ± 0.0%[1]	0.00 ± 0.0%[1]	_[2]	_[2]
Pueblo	0.38 ± 0.3%	0.03 ± 0.0%	0.06 ± 0.0%	0.00 ± 0.0%[1]	8.9 ± 3.2%

[1] Estimated sequence divergence calculated based only on *Eco*RI fragment pattern.

[2] Sequence divergence could not be calculated due to 0% fragment sharing.

trate dehydrogenase-1. At these three polymorphic loci, allelic frequencies were almost fixed for the common allele in the Cherry Creek population and fixed for the common allele in the McConaughy population, but were intermediate (0.31-0.69) in the other three populations (Table 3). Genetic distances (Nei, 1978) were D = 0.00-0.07 among populations based on all 12 loci.

COMPARISONS OF POPULATION STRUCTURE

Similarities among populations were estimated with five restriction enzyme fragment patterns (*Ase*I, *Eco*RI, *Hae*III, *Hin*dIII, and *Hin*fI) for mtDNA data and with all 12 loci for isozyme data. Fig. 2 is the phenogram produced by the UPGMA clustering algorithm based on estimated sequence divergence for mtDNA data and on Nei's I for allozyme data. Both the mtDNA RFLP data and allozyme data revealed genetic differentiation to distinguish the Cherry Creek and McConaughy populations in the South Platte River drainage from the populations in the Republican drainage (Flagler population) and Arkansas River drainage (Pueblo population and Colorado Fuel and Iron population).

DISCUSSION

Historically, the giant floater was probably never found in the main channels of the Arkansas River, the South Platte River, and the Republican River. It is possible that the Platte River, with its shallow channels, rapid flow, shifting sand and gravel bottoms, and drastic changes in flow never provided a suitable habitat to support the mussels (Roedel, 1990; Peyton and Maher, 1992). Furthermore, a substantial amount of water is being taken out of the Platte River for irrigation in summer (Peyton, pers. comm.), thus further reducing the summer flow and increasing the flow-level changes among seasons. Because most of the main channels of the Republican River are completely dry during the summer season, they are inaccessible to mussels. During most years, the Arkansas River has a continuous flow from Pueblo, Colorado, to Garden City, Kansas. During some drought years, however, the main-stem of the Arkansas River near the Colorado-Kansas border has been known to have no surface flow.

Along the river drainages, oxbows, sloughs, and springs provide suitable habitats for mussels, but no populations were found in those areas. It is possible that industrial effluents, agricultural impounding, and irrigation diversions have periodically reduced fish stocks necessary to sustain and disperse glochidia.

Historically, the density of mussels reached 6-12 per square foot (Brandauer and Wu, 1978). Our survey of populations revealed that all were at densities of less than 0.01 mussels per square foot. This density is only approximate; it is calculated from a coarse estimate of the area searched

Table 3. Allele frequencies at three polymorphic loci among populations in *Pyganodon grandis*. (IDH-1, isocitrate dehydrogenase-1; PGM-1, phosphoglucomutase-1; PGM-2, phosphoglucomutase-2).

Locus	Allele	Cherry Creek (N = 12)	Colorado Iron and Fuel (N = 15)	Flagler (N = 16)	McConaughy (N = 8)	Pueblo (N = 12)
IDH-1	100	0.98	0.53	0.69	1.00	0.50
	80	0.02	0.47	0.31	0.00	0.50
PGM-1	107	0.05	0.50	0.42	0.00	0.42
	100	0.95	0.50	0.58	1.00	0.58
PGM-2	100	0.91	0.40	0.63	1.00	0.37
	80	0.09	0.60	0.37	0.00	0.63

and the number of live individuals located. Although pollution, fluctuation in water levels, and periodic decimation of fish stock can all contribute to the decline of mussels, it is not known which factor is the most important.

The paternal and maternal mtDNA RFLP data and isozyme data revealed an insignificant amount of genetic differentiation among the Pueblo, Colorado Fuel and Iron, and Flagler populations, but revealed different degrees of genetic differentiation between the above three populations and the Cherry Creek and McConaughy populations. Genetic similarity between Pueblo and Colorado Fuel and Iron Reservoir No. 2 populations is explained by founding of the Pueblo population from the Colorado Fuel and Iron Reservoir No. 3 population. Colorado Fuel and Iron Reservoirs No. 2 and No. 3 are adjacent to each other and the fish can move freely from Reservoir No. 3 to Reservoir No. 2. The genetic differentiation between the Arkansas River drainage populations (Pueblo population, Colorado Fuel and Iron population) and the South Platte River drainage population (Cherry Creek population, McConaughy population) can be explained easily by a lack of gene flow between these two drainage systems. However, it is puzzling that the Republican River drainage population is more similar to the Arkansas River drainage populations than to the South Platte River drainage population. Both the South Platte and Republican Rivers discharge into the Missouri River, with the Arkansas River discharging further south directly into the Mississippi River. Fish carrying glochidia could move among these three drainage systems. Therefore, it was expected that the South Platte and the Republican populations would be more closely related to each other than to the Arkansas drainage populations. However, for many years the Colorado Division of Wildlife has stocked various fishes in the reservoirs, and fish can carry glochidia from six days to six months. Thus, the similarity between the Arkansas and the Republican River drainage populations could be due to unintentional mussel transfer through fish stocking. One question arises immediately: Were either or both of these mussel populations in the South Platte and the Arkansas River drainage established by accidental transfer? Available data suggest that the Cherry Creek population represents the original population in terms of genetic structure in the South Platte River drainage system for the following two reasons. First,

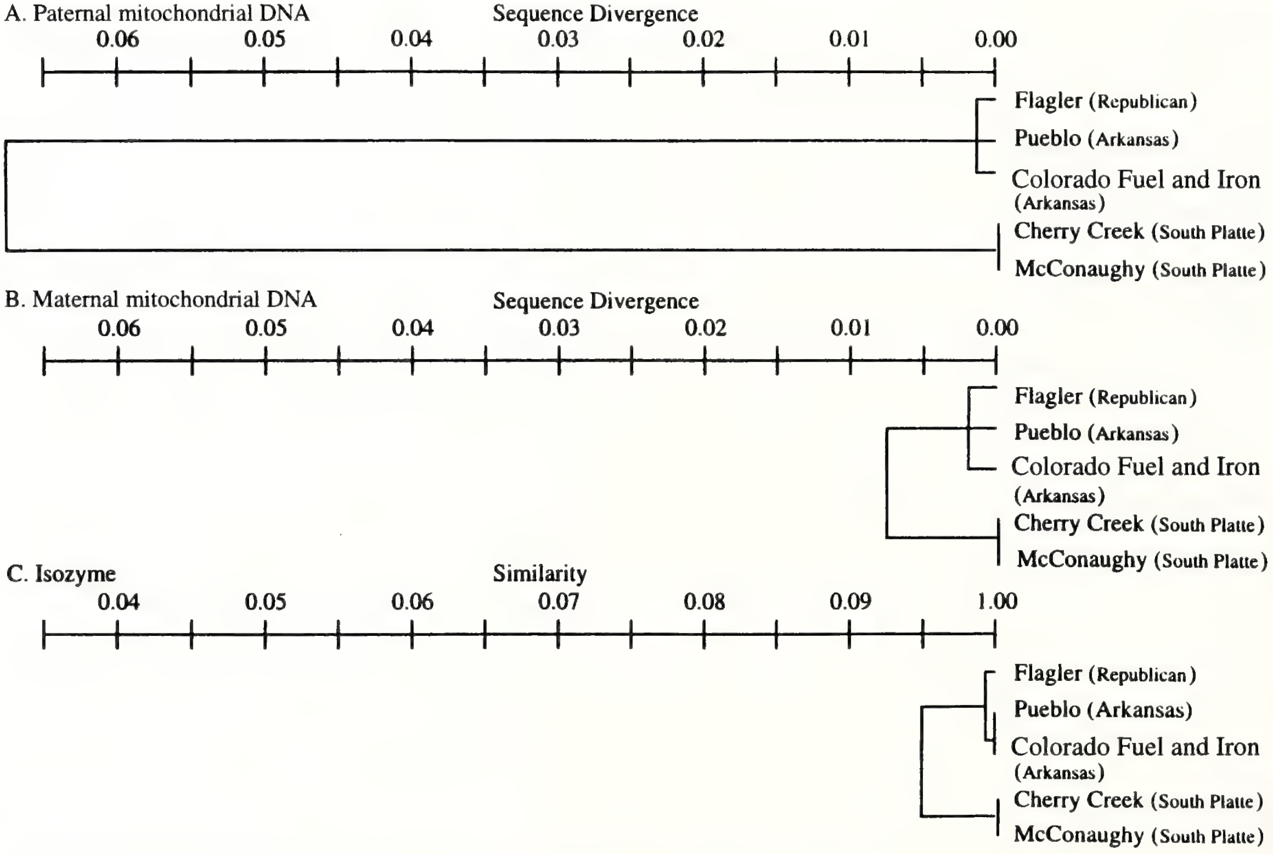

Fig. 2. Phenograms constructed by using UPGMA clustering algorithm for paternal mtDNA (A), maternal mtDNA (B), and isozyme data (C).

the earliest record of the giant floater was reported by Cockerell in 1889 in Black Wolf Creek in Yuma County in the South Platte River drainage system. This record indicated that native mussels existed in the South Platte River drainage in Colorado before the Colorado Division of Wildlife was established. Second, we were also able to sample one natural mussel population, the slough near Lake McConaughy, located in the South Platte River drainage system in Nebraska. Both mtDNA and isozyme data suggest that the McConaughy population is similar to the Cherry Creek population. Additionally, there is no historical natural population record for the Arkansas River drainage system to verify the natural occurrence of mussels in the Arkansas River. Fausch *et al.* (1985) reported shells of the giant floater from an eroding bank at the mouth of Van Bremer Arroyo within 500 m of the Purgatoire River, a tributary of the Arkansas River in southeastern Colorado. Herrmann (unpubl.) collected shell fragments from the Van Bremer site. However, to date no extant populations have been found in the Purgatoire River. These observations raise the possibility that mussels were in the Arkansas River and its tributaries, but disappeared before the geographic surveys (Wu, 1989) of the giant floater.

The gender-specific mtDNA's of the giant floater exhibit discordant degrees of geographic variation. Among populations, females are similar (0-0.5%) but the mitochondrial sequences of males differ by 11%. Although the mtDNA haplotypes of females are shared among localities, the mtDNA haplotypes of males are diagnostically different between the drainage systems. The virtual lack of geographic differentiation of maternal mtDNA and the high degree of differentiation for paternal mtDNA presents an unprecedented and intriguing puzzle. A survey of additional populations and geographic areas and physiological studies of the maternal type and paternal type mitochondrial genomes are needed to understand the evolutionary forces producing these discordant degrees of geographic variation. Surveys throughout the range of the giant floater are needed to determine the full extent of differentiation of the mitochondrial forms, and to determine whether the disparity in the level of differentiation of the maternal and paternal forms is found in other parts of the range.

MANAGEMENT

In our study, both the mtDNA RFLP data and allozyme data revealed genetic differentiation to distinguish the Cherry Creek and McConaughy populations in the South Platte River drainage from the populations in the Republican and Arkansas River drainage. The male mtDNA haplotypes are diagnostic for these drainages, indicating that there has not been any gene flow between these drainages for a very long time. We have no information to indicate that the differentiation is neutral or adaptive, but the long period of genetic isolation of these drainages provided ample opportunity for adaptive differentiation, if not in the male mtDNA, then in some other portion of the genome. Given this opportunity for the evolution of adaptive differentiation, it would be imprudent to exchange individuals between these drainage systems. Precautions should be taken to avoid gene flow among drainages.

Due to the genetic differentiation between drainage systems, we recommend that the Cherry Creek population should serve as the source population for the restoration of the giant floater in the South Platte River drainage system. The Pueblo, Colorado Fuel and Iron, and Flagler populations are all suitable as source populations for the restoration in the Arkansas and Republican River drainage systems.

ACKNOWLEDGMENTS

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LITERATURE CITED

Avise, J. C. 1994. *Molecular Markers, Natural History and Evolution.* Chapman and Hall, New York. 511 pp.

Avise, J. C. and W. S. Nelson. 1989. Molecular genetic relationships of the extinct dusky seaside sparrow. *Science* 243:646-648.

Brandauer, N. and S.-K. Wu. 1978. The freshwater mussels (family Unionidae). *Natural History Inventory of Colorado* 2:41-60.

Burch, J. B. 1975. *Freshwater Unionacean Clams (Mollusca: Pelecypoda) of North America.* Malacological Publications, Hamburg, Michigan. 176 pp.

Cockerell, T. D. A. 1889. Preliminary remarks on the molluscan fauna of Colorado. *Journal of Conchology* 6:60-65.

Fausch, K. D., D. L. Miller, B. D. Rosenlund, and L. D. Zuckerman. 1985. Aquatic organisms and habitat of the Purgatoire River and tributaries, U. S. Army, Piñon Canyon Maneuvers Site, Colorado. *U. S. Fish and Wildlife Service Report.* 76 pp.

Fuller, S. L. H. 1974. Clams and mussels (Mollusca: Bivalvia). *In: Pollution Ecology of Freshwater Invertebrates,* C. W. Hart, Jr. and S. L. H. Fuller, eds. pp. 215-273. Academic Press, New York.

Herrmann, S. J. and J. R. Fajt. 1985. Additional Colorado records of *Anodonta grandis grandis* Say (Bivalvia: Unionidae). *The Nautilus* 99:93-100.

Hoeh, W. R. 1990. Phylogenetic relationships among eastern North American *Anodonta* (Bivalvia: Unionidae). *Malacological Review*

23:63-82.

Hunter, R. D. and J. F. Bailey. 1991. Colonization of soft substrata by zebra mussels: role of and costs to native bivalves. *Journal of Shellfish Research* 10:249-250.

Hunter, R. D. and J. F. Bailey. 1992. *Dreissena polymorpha* (zebra mussel): colonization of soft substrata and some effects on unionid bivalves. *The Nautilus* 106(2):60-70.

Karl, S. A. and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science* 256:100-102.

Liu, H.-P., J. B. Mitton, and S.-K. Wu. 1996. Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis grandis*. *Evolution* 50(2):952-957.

Miller, J. C. 1991. RESTSITE: a phylogenetic program that sorts raw restriction data. *The Journal of Heredity* 82(3):262-263.

Mitton, J. B. 1994. Molecular approaches to population biology. *Annual Review of Ecology and Systematics* 25:45-69.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.

Peyton, M. M. and J. L. Maher. 1992. *Bivalves in Central Nebraska Irrigation Systems and Associated Reaches of the Platte River.* Unpublished manuscript, Central Nebraska Public Power and Irrigation District. 9 pp.

Roedel, M. D. 1990. Unionid molluscs in the Big Bend reach of the Platte River, Nebraska. *Prairie Naturalist* 22(1):27-32.

Skibinski, D. O. F., C. Gallagher, and C. M. Beynon. 1994. Mitochondrial DNA inheritance. *Nature* 368:817-818.

Stolzenburg, W. 1995. Scorecard of scarcity. *Nature Conservancy* May/June 1995:6.

Swofford, D. L. and R. B. Selander. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72:281-283.

Trdan, R. J. and W. R. Hoeh. 1982. Eurytopic host use by two congeneric species of freshwater mussel (Pelecypoda: Unionidae: *Pyganodon). The American Midland Naturalist* 108(2):381-388.

Williams, J. D., M. L. Warren, Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1992. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* 18(9):6-22.

Wu, S.-K. 1989. Colorado freshwater mollusks. *Natural History Inventory of Colorado* 11:1-117.

Zouros, E., A. O. Ball, C. Saavedra, and K. R. Freeman. 1994. Mitochondrial DNA inheritance. *Nature* 368:818.

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Research Note

A method for preparing freshwater mussels (Mollusca: Unionoida) for anatomical study

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Abstract: A method is described for preparing freshwater mussels for anatomical study. Narcotization of specimens with menthol according to a chronological protocol is followed by hypodermic injection with formalin. Formalin injection pressurizes vascular spaces and fixes tissues simultaneously. This results in preserving the animal in a life-like posture.

Key words: freshwater mussels, narcotization, preservation, methods

The increasing need to study the biology of freshwater mussels (Unionoida) is prompted by the rapid decline of a large number of the species throughout the world. The crisis is probably best documented in North America where 113 species, approximately 38% (Bogan, 1993) to 55% (Master, 1990) of the fauna are considered to be rare and declining or nearly extinct. Estimates of extinct species range from 12 (Williams and Novak, 1986) to 19 (Bogan, 1993) to 21 (Williams *et al.*, 1992), approximately 4 to 7% of the fauna. These estimates could even go higher based on United States Fish and Wildlife Service investigations (P. Hartfield, USFWS, pers. comm.). Considering the endangered status of many of the remaining species, a greater sense of restraint and justification is now required when animals are permanently removed from the population for post-mortem studies and deposition in museum collections. The ability to gather all sorts of biological information necessitates the removal and killing of animals for various research projects. However, the current concern over the plight of many unionoid mussels should make it unnecessary to argue that any specimen taken from the wild should have its potential use maximized. The past practice of retaining specimens for their shell characters only is outmoded, wasteful, and altogether unneccessary.

Coney (1993) discussed a number of methods of anesthestization and monitored several aspects of tissue response to preservation. His study attempted to determine the best method to maximize retrievability of information of all parts of the soft anatomy. Thus, in addition to producing the greatest extension of mantle tissues, the nature of the epithelial surfaces, including the cilia, should also be of the best quality to receive a high evaluation for a particular technique. Coney's (1993) review of various reagents and applications considered their effect on mussels of the family Unionidae for the most part. One of the many chemicals tested by Coney was menthol. He indicated that he had "not found menthol to be effective in the anesthetization of unionids." He further reviewed some findings in published reports regarding the efficacy of menthol, the results of which seemed to be inconclusive.

The method described here was first experimentally used during a study on freshwater mussels for which anatomical data was sought, particularly details of mantle structure and pigmentation (Smith, 1982). In the time following that study, the method has been perfected and a large number of northeastern Atlantic coast and Interior Basin species have been subjected to the process. A recent opportunity to work with the United States Fish and Wildlife Service (USFWS) (National Biological Survey) staff in the Gainesville, Florida, field laboratory, enabled trials with additional species restricted to Gulf coast drainages, some of which are notoriously difficult to narcotize.

MATERIALS

The equipment and supplies required are listed in Table 1. The principal narcotizing agent used is menthol. I have had excellent success with menthol with a number of freshwater invertebrate taxa, including cnidarians, ento-

Table 1. Equipment and supplies for preparing specimens.

menthol, powdered
plastic containers with lids or covers (4-8 l in capacity)
rubber gloves
60 cc (3 oz) syringe
1.5" #20 gauge needles (#19-23 gauge also acceptable)
3.5" spinal needle
stock formalin solution (10%)
large white pan
probe

procts, ectoprocts, turbellarian worms, gastropods, and unionoid mussels. Success in using menthol in especially narcotizing freshwater mussels is dependent on the application schedule and dosage, which is described below.

Menthol should be in powder form, as available from Sigma Chemical Company (St. Louis, Missouri; Catalog 1995, catalog number M 2258), or Aldrich Chemical Company (Milwaukee, Wisconsin; Catalog 1995, catalog number M278-0). A 100 gm bottle will supply enough material for several years use. The plastic containers should be clear so that specimens can be observed and each must be provided with a cover that properly seals. Containers can be purchased from department stores under any number of brand names and descriptions. Syringes are available from various veterinary supply houses.

THE METHOD

Freshly collected specimens must be maintained in water from the collection locality and transferred with the water to the plastic containers. Do not crowd the specimens; about 2-3 cm should be allowed between individual mussels. Enough water can then be added or removed to bring the level to about 2 cm above the largest specimen. Apply menthol until a very light film is formed on the surface of the water. (Note: If the stock menthol has been subjected to moisture it will form large clumps. These must be ground to powder before use.) Seal the container and place some object of weight on the cover to insure a good seal. At this point, allow the specimens to stand undisturbed for 24 hr. (The container is affected by room temperature and this 24 hr rest period is based on room temperatures of 20-22° C). After the 24 hr period, check the specimens. Most of the menthol will be dissolved and a strong odor of menthol should be detectable. The specimens will have begun to gape open, the extent of which is dependent upon the species being treated. Typically, a small portion of the foot will be extended.

Add a small quantity of menthol, about half as much as used originally, to the water and reseal the container. Visual inspection of specimens in the sealed container should now become more frequent to monitor gaping of the valves. The mantle will become slightly more relaxed with time, although the foot will hardly extend farther out of the shell. After several more hours have passed, periodic checks for relaxation can be performed. Gently rub a probe along the leading edge of the foot. Rapid contraction indicates that distal nerves are still sensitive. When the response is a very slow and localized contraction, or is nonexistent, then the animal is narcotized. Waiting an additional period of time, 1-2 hrs, will insure that full relaxation has occurred. It is essential that death does not occur because animals will not survive long in a relaxed state and during death, a slow, involuntary, and irreversible retraction of tissue takes place.

When relaxed, gently remove the animal from the water and hold it upside down (Fig. 1). Insert a syringe filled with 10% formalin through the supra-anal region of the mantle and into the animal as far as the pericardial region just behind the umbo (Fig. 2). Inject formalin slowly into the pericardium. This part of the procedure should be performed under a hood or in a well-ventilated area. When fully relaxed, the injection pressurizes the vascular and hemocoelic spaces and forces the relaxed mantle and foot

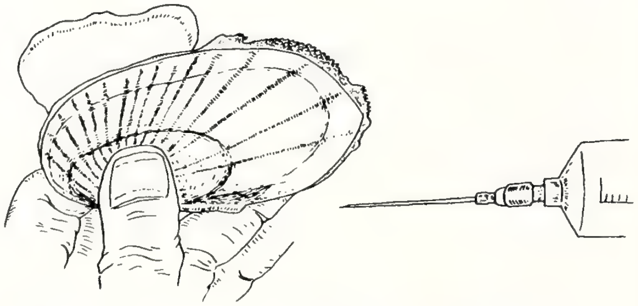

Fig. 1. Method of insertion of hypodermic in a mussel specimen.

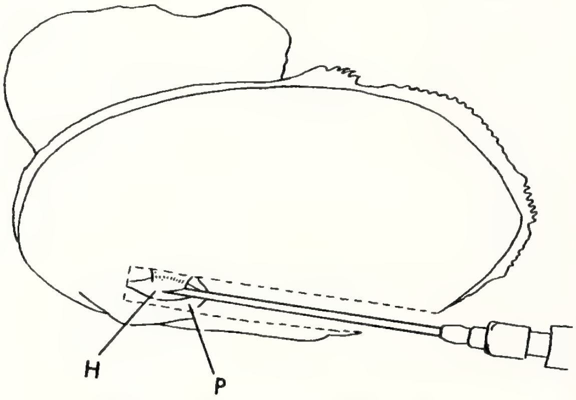

Fig. 2. Proper location of needle during formalin injection. (H = heart; P = pericardium).

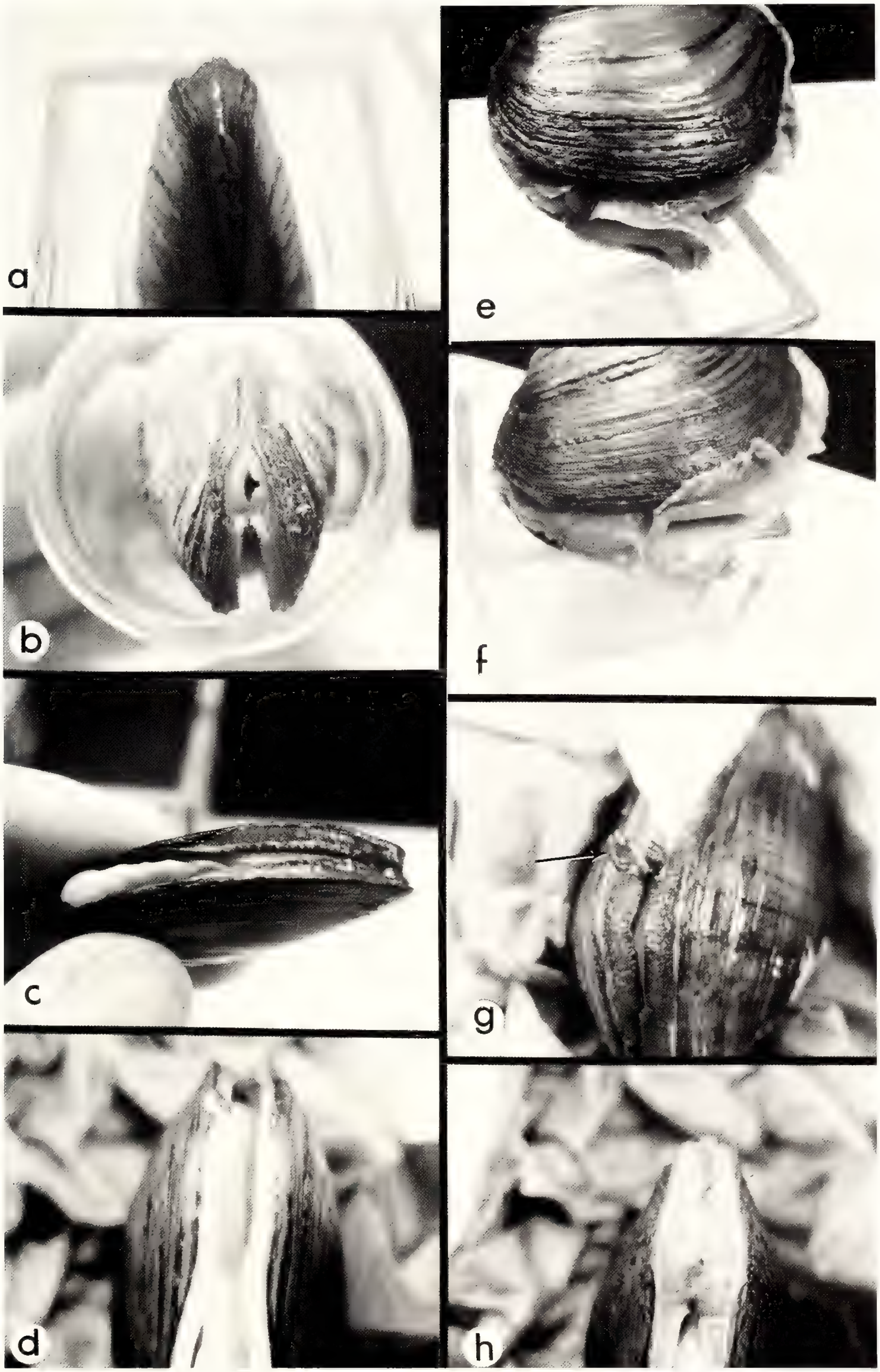

Fig. 3. Examples of mantle perservation in several species treated by the described method. (a) *Margaritifera margaritifera* (Linné, 1758); (b) *Anodontoides ferussacianus* (Lea, 1834); (c) *Ligumia nasuta* (Say, 1817); (d) *Potamilus alatus* (Say, 1817); (e-f) *Lampsilis ovata* (Say, 1817); (g) *Lampsilis radiata* (Gmelin, 1791) (s.l.), dark form, arrow denotes flap; (h) *L. radiata* (s.l.), light form. Specimens b and d-h are from Lake Champlain drainage; a and c are from Connecticut River drainage.

tissues to expand. When the mantle can expand no further, remove the needle. Place the animal in a dry sink or container (such as a large white pan) to allow excess formalin and fluid to drain. Do not immerse the animal in formalin or any other fixative or preservative at this time. Repeat the injections two or three times at 2-3 min intervals. After 5-10 min, the animal should be dead. Perform one more injection to full extension and immediately immerse the specimen in fixative. Subsequent handling, processing, and storage procedures should follow standard museum practices.

Extreme care should be taken to insure narcotization and if patient application of the method is followed, the procedure will produce excellent specimens for study (Fig. 3). As with any procedure, "practice makes perfect" and preliminary trials with common species will give the investigator useful experience, especially with the narcotization phase. Results have shown that after 15 yr of museum storage, major pigment patterns and structures are still discernible, although coloration can be lost. Advantages of this method are that it involves relatively inexpensive and readily available equipment and supplies and requires no federal or state permits other than those required for collecting specimens. If ethanol is preferred over isopropanol as a final storage preservative, a permit will be required.

LITERATURE CITED

Bogan, A. E. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. *American Zoologist* 33:599-609.

Coney, C. C. 1993. An empirical evaluation of various techniques for anesthetization and tissue fixation of freshwater Unionoida (Mollusca: Bivalvia), with a brief history of experimentation in molluscan anesthetization. *The Veliger* 36:413-424.

Master, L. 1990. The imperiled status of North American aquatic animals. *Biodiversity Network News* 3:1-2, 7-8.

Smith, D. G. 1982. The zoogeography of the freshwater mussels of the Taconic and southern Green Mountain region of northeastern North America (Mollusca: Pelecypoda: Unionacea). *Canadian Journal of Zoology* 60:261-267.

Williams, J. D. and R. M. Novak. 1986. Vanishing species in our own backyard: extinct fish and wildlife of the United States and Canada. *In: The Last Extinction,* L. Kaufman and K. Mallory, eds. pp. 107-139, MIT Press, Cambridge.

Williams, J. D., M. L. Warren, Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1992. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* 18:6-22.

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Annotated catalog of malacological meetings, including symposia and workshops in malacology

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Abstract: As a much needed bibliographic tool, an annotated catalog is given of the symposia and workshops that have been held at malacological and generalist meetings over the past six decades, together with their resulting publications. Particularly detailed emphasis is given to the meetings of Unitas Malacologica, the American Malacological Union, and the Western Society of Malacologists.

This paper catalogs the symposia and workshops that have taken place at malacological meetings over the last six decades, as well as those that have occurred at other venues. The publications that resulted from these meetings and symposia are listed, chiefly because this information can be difficult for researchers to obtain. This catalog is not complete, and it emphasizes natural history and systematics. We have not endeavored to document every malacological meeting, particularly those before 1930, and those of European and Asian national societies that do not seem to have had symposia or have resulted in publications. Moreover, we have not thoroughly covered meetings on shellfisheries, mariculture, agricultural or other pests, and mollusk-borne diseases, nor those of shell-collectors' groups. These organizations might want to provide their own listings for the historical record. A listing of symposia on cephalopods is in preparation by F. G. Hochberg; as a result, we list only those cephalopod symposia occurring at Unitas Malacologica, the American Malacological Union, or other meetings already covered in detail. We have not covered meetings held or publications issued after 1995.

Our catalog is most comprehensive and detailed for the meetings of the Unitas Malacologica, the American Malacological Union (AMU), and the Western Society of Malacologists (WSM). These three groups are listed first, followed by other groups in alphabetical order, and lastly by symposia and workshops not part of a series of malacological group meetings. In 1948, the American Malacological Union established a Pacific Division, which began to meet each year in the western United States. In 1968, this division was replaced by an independent Western Society of Malacologists; that year, the AMU-PD and WSM meetings were held concurrently. Commencing with the 1987 meeting, AMU abstracts were no longer published separately and were only issued at the meeting. However, at joint AMU-WSM meetings, the WSM Annual Report contains abstracts of many AMU papers.

We would greatly appreciate additional information on previous and future meetings and symposia to update this publication for eventual addenda. One benefit of this catalog is to draw attention to topics neglected at past meetings.

We define a symposium as a pre-planned program of related papers, most or all invited. This is as opposed to a set of contributed, non-solicited papers grouped by topic in formulating a meeting agenda. Symposia generally have one or more organizers; related-paper sessions have only a chair. Obviously, there is not a sharp distinction between these categories. The symposia listed here commence with one at the 1941 AMU meeting and include one that had only four participants. Workshops are generally several-day-long study sessions, often including field or laboratory work, and frequently resulting in special publications.

In the following catalog, the year of the meeting is given, followed by the congress or meeting number (if applicable), the location and dates of the meeting, the sponsoring organization(s) and meeting organizers (if applicable), and the resulting publication(s), first for the meeting as a whole, then for any symposia or workshop publications. The symposia publications are full-length papers or proceedings unless specifically indicated as only abstracts or a report. Proceedings are assumed to be edited by the meeting organizer unless otherwise indicated. Meeting abstracts are generally not listed if there was a subsequently issued proceedings volume. The documented dates of publication of these works have been included when known.

American Malacological Bulletin, Vol. 13(1/2) (1996):129-148

The month dates on the covers of some of the early volumes of the *American Malacological Bulletin* are known to contain errors and are not given here.

European Malacological Congresses/Unitas Malacologica

- 1962 1 London, England, United Kingdom, 17-21 September.
  - L. R. Cox and J. F. Peake, eds., 1965, *Proceedings*, Conchological Society of Great Britain and Ireland, and Malacological Society of London, viii + 266 pp.
- 1965 2 Copenhagen, Denmark, 10-14 August.
  - *Malacologia* 5(1): vi + 93 pp., G. H. Peterson and J. Knudsen, eds., 1966 (31 December).
  - Malacology and Parasitology – Henning Lemche.
    - *Malacologia* 5(1):15-36 [abstracts].
- 1968 3 Vienna, Austria, 2-6 September.
  - *Malacologia* 9(1): viii + 338 pp., O. E. Paget, ed., 1970 (6 June).
  - Molluscs as Parasites or their Transmitters, H. Mohorst, John B. Burch and J. A. v. Eeden, organizers.
    - *Malacologia* 9(1):25-44 [abstracts and papers].
- 1971 4 Geneva, Switzerland, 7-11 September.
  - *Malacologia* 14(1-2): vi + 478 pp., E. E. Binder, ed., 1974 (3 January).
- 1974 5 Milan, Italy, 3-7 September.
  - *Malacologia* 16(1): vi + 331 pp., F. Toffoletto, ed., 1977 (12 August).
  - Polymorphism in Gastropoda, C. B. Goodhart, organizer.
    - *Malacologia* 16(1):9-47.
    - *Littorinid Tidings* 2:19-20, 1974 (September) [abstracts on Littorinidae].
- 1977 6 Amsterdam, The Netherlands, 15-20 August.
  - Amsterdam (Institute of Taxonomic Zoology), A. C. van Bruggen, ed., 152 pp., 1977 [abstracts].
  - *Malacologia* 18(1-2): xxii + 622 pp., A. C. van Bruggen, ed., 1979 (18 May).
  - S. van der Spoel, A. C. van Bruggen, and J. Lever, eds., 1979, *Pathways in Malacology*, Bohn, Scheltema, and Holkema Utrecht, 295 pp. [invited lectures].
- 1980 7 Perpignan, France, 31 August - 7 September.
  - *Haliotis* 10(2): 190 + x pp., J. M. Gaillard, ed., 1980 [abstracts].
  - *Malacologia* 22(1-2): xxx + 765 pp., J. M. Gaillard *et al.*, eds., 1982 (24 June).
  - Pathology and Parasitology, Constantin Vago and Claude Combes, organizers.
    - *Malacologia* 22(1-2):1-102.
  - Sexuality and Reproduction, Joos Joosse, Pierre Lubet and Norman Runham, organizers.
    - *Malacologia* 22(1-2):103-223.
  - Calcium and Skeletal Structures, Winfried Haas and Gottfried Krampitz, organizers.
    - *Malacologia* 22(1-2):225-339.
  - Growth and Productivity, Jacques Daguzan and Bernard Salvat, organizers.
    - *Malacologia* 22(1-2):341-401.
  - Evolution and Adaptive Radiation, George M. Davis, organizer.
    - *Malacologia* 21(1-2): 430 pp., 1981 (8 December).
- 1983 8 Budapest, Hungary, 24 August - 4 September.
  - Hungarian Natural History Museum, Budapest, L. Pinter, ed., 166 pp., 1983 [abstracts].
  - Hungarian Natural History Museum, Budapest, L. Pinter, ed., xviii + 342 pp., 1986 [proceedings].
  - Biogeography of Land and Freshwater Mollusks, Alan Solem, organizer.
    - Alan Solem and A. C. van Bruggen, eds., 1984, *World-wide Snails*; *biogeographic studies on non-marine Mollusca,* Brill, Leiden, x + 289 pp.
  - Sphaeriidae, J. G. J. Kuiper, organizer.
    - Abstracts scattered in proceedings.
  - Applied Freshwater/Terrestrial Malacology and Parasitology, Thomas K. Kristensen, organizer.
    - Abstracts scattered in proceedings.
  - Quaternary Malacology and Fauna History, Endre Krolopp, organizer.
    - Abstracts scattered in proceedings.
  - E[uropean] I[nvertebrate] S[urvey] Session.
    - Proceedings:305-337.
- 1986 9 Edinburgh, Scotland, United Kingdom, 31 August - 6 September.
  - Edinburgh, National Museums of Scotland, D. Heppell, ed., 104 pp. [abstracts].
  - Unitas Malacologica, Leiden, E. Gittenberger and J. Goud, eds., 414 pp., 1992 [proceedings].
  - Evolutionary Biology of Opisthobranchs, Malcolm Edmunds, organizer.
    - *Malacologia* 32(2):203-327, 1991 (7 June).

Prosobranch Phylogeny, Winston F. Ponder, organizer.
Malacological Review, supplement 4: vi + 346 pp., 1988 (20 December).
The Bivalvia, Brian Morton, organizer.
Brian Morton, ed., 1990, *The Bivalvia; a memorial symposium in honour of Sir Charles Maurice Yonge,* Hong Kong University Press, viii + 355 pp.
Reports of the Specialist Group Workshop E.I.S. [European Invertebrate Survey].
Apex 6(3-4):87-100, 1986 (December).
Applied Malacology.
Journal of Medical and Applied Malacology 4:1-178, 1992.
Conservation Biology of Molluscs.
E. Alison Kay, ed., 1995, The conservation biology of molluscs. Proceedings of a symposium held at the 9th International Malacological Congress, Edinburgh, Scotland, 1986, *International Union for the Conservation of Nature, Species Survival Commission, Occasional Paper* 9: iii + 81 pp.

1989 10 Tübingen, West Germany, 27 August - 2 September.
Tropenmedizinischen Institüt der Universität Tübingen, C. Meier-Brook, ed., ii + 287 pp., 1989 [abstracts].
Baja, Hungary, 2 volumes: vi + 636 + x pp., C. Meier-Brook, ed., 1992 [proceedings].
Biology and Evolution of Toxoglossan Gastropods, John D. Taylor, organizer.
John D. Taylor, ed., 1990 (30 November), Biology and evolution of toxoglossan gastropods, *Malacologia* 32(1):1-87.
E.I.S. [European Invertebrate Survey] Symposium.
Mitteilungen der Deutschen Malakozoologischen Gesellschaft 48:1-54, J. H. Jungbluth, ed., 1991.
Workshop on Unionoidea, J. H. Jungbluth, organizer.
Archiv für Hydrobiologie [in press].
Workshop on Hydrobioid Phylogeny, Robert Hershler, organizer.

1992 11 Siena, Italy, 30 August - 5 September.
University of Siena, F. Giusti and G. Manganelli, eds., 536 pp., 1992 (June) [abstracts].
Workshop on Systematic[s] and Ecology in the Opisthobranchia, R. Cattaneo-Vietti, organizer.
Bollettino Malacologico 29(5-8):109-209, 1993 (30 November).
Molecular Techniques and Molluscan Phylogeny, M. G. Harasewych and Simon Tillier, organizers.
M. G. Harasewych and Simon Tillier, eds., 1994 (11 August), Molecular techniques and molluscan phylogeny, *The Nautilus*, supplement 2: ii + 174 pp.
Molluscan Palaeontology, A. W. Janssen and R. Janssen, organizers.
Scripta Geologica, special issue 2: 436 pp., 1993 (December).
Alan Solem Memorial Symposium on the Diversity and Conservation of the Mollusca, A. C. van Bruggen, Susan M. Wells, and Theo. C. M. Kempermann, organizers.
A. C. van Bruggen, Susan M. Wells, and Theo. C. M. Kempermann, eds., 1995, *Biodiversity and Conservation of the Mollusca,* Proceedings of the Alan Solem Memorial Symposium on the Biodiversity and Conservation of the Mollusca, Backhuys, Leiden, xi + 228 pp.
Molluscan Behaviour, Guido Chelazzi, Paolo Della Santina, and Roger N. Hughes, eds.
Ethology, Ecology and Evolution (Firenze) 6(1):37-124, 1994.

1995 12 Vigo, Spain, 3-9 September.
Vigo, Instituto de Investigaciones Marinas, Angel Guerra, Emilio Rolán, and Francisco Roicha, eds., 538 pp., 1995 (August) [abstracts].
Symposia:
Freshwater Molluscs, M. A. Ramos, W. Heard, and R. Araujo, organizers.
Malacological Review or *Malacologia* [in preparation].
Functional Morphology of Cephalopods, A. Guerra, S. v. Boletzky, and P. Fioroni, organizers.
Journal of Molluscan Studies [in preparation].
Endemism in the Marine Realm, S. Gofas, organizer.
Haliotis [in preparation].
Molluscan Pathology, H. Grizel and A. Figueras, organizers.
Aquatic Living Resources [in preparation].
Ecology of Molluscs, J. D. Ros, organizer.
Scientia Marina [in preparation].

Molluscan Evolution and Fossil Records, J. Martinell, and K. Bandel, organizers.
Iberus [in preparation].

Workshops:
Medical and Applied Malacology, J. B. Burch and Y. Manga, organizers.
Journal of Medical and Applied Malacology [in preparation].
Malacology Applied to Food Production, L. J. Elmslie, organizer.
Systematics and Ecology of Opisthobranchs, R. Cattaneo-Vietti, V. Urgorri, and J. F. Troncoso, organizers.
ETI and CLEMAM Data Base Information, K. E. Hoagland and E. Platts, organizers.

American Malacological Union [AMU]

1931 1 Philadelphia, Pennsylvania, U. S. A., 30 April - 2 May.
The Nautilus 45(1):1-5, 1931 (13 July).
1932 2 Washington, D. C., U. S. A., 26-28 May.
The Nautilus 46(1):1-3, 1932 (23 July).
1933 3 Cambridge, Massachusetts, U. S. A., 25-27 May.
The Nautilus 47(1):37-44, 1933 (16 June).
1934 4 Stanford, California, U. S. A., 25-28 June.
AMU Report for 1934: 12 pp., 1934 (1 August).
1935 5 Buffalo, New York, U. S. A., 27-29 June.
AMU Report for 1935: 10 pp., 1935.
1936 6 St. Petersburg, Florida, U. S. A., 21-24 April.
AMU Report for 1936: 11 pp., 1936 (post-1 September).
1937 7 Ann Arbor, Michigan, U. S. A., 3-5 August.
AMU Report for 1937: 16 pp., 1938 (post-1 January).
1938 8 Havana, Cuba, 1-6 August.
AMU Report for 1938: 20 pp., 1939 (post-1 January).
1939 9 Toronto, Ontario, Canada, 20-23 June.
AMU Report for 1939: 16 pp., 1939 (November).
1940 10 Philadelphia, Pennsylvania, U. S. A., 7-21 June.
AMU Report for 1940: 18 pp., 1940 (December).
1941 11 Rockland, Maine, U. S. A., 26-29 August.
AMU Report for 1941: 44 pp., 1942 (January).
Methods of Collecting and Preserving Mollusca, Frank C. Baker, organizer.
AMU Bulletin for 1941 2:5-37.
1942-1943 - no meetings.
AMU News Bulletin and Annual Report for 1943: 24 pp., 1944 (January).
1944-1945 - no meetings.
AMU News Bulletin and Annual Report for 1944-1945: 21 pp., 1945 (October).
1946 12 Washington, D. C., U. S. A., 14-16 August.
AMU News Bulletin and Annual Report for 1946: 22 pp., 1947 (April).
1947 13 Pacific Grove, California, U. S. A., 18-21 June.
AMU News Bulletin and Annual Report for 1947: 32 pp., 1947 (December).
1948 14 Pittsburgh, Pennsylvania, U. S. A., 25-27 August.
AMU News Bulletin and Annual Report for 1948: 36 pp., 1949 (March).
1 Pacific Division, Los Angeles, California, U. S. A., 10-11 April.
1949 15 Coral Gables, Florida, U. S. A., 16-18 June.
AMU News Bulletin and Annual Report for 1949: 36 pp. 1949 (November).
2 Pacific Division, Long Beach, California, U. S. A., 14-16 June.
1950 16 Chicago, Illinois, U. S. A., 14-16 June.
AMU News Bulletin and Annual Report for 1950: 39 pp., 1950 (December).
3 Pacific Division, Santa Barbara, California, U. S. A., 7-9 April.
1951 17 Buffalo, New York, U. S. A., 22-24 August.
AMU News Bulletin and Annual Report for 1951: 40 pp., 1951 (December).
4 Pacific Division, Oakland, California, U. S. A., 22-24 June.
1952 18 Boston, Massachusetts, U. S. A., 20-22 August.
AMU Annual Report for 1952: 44 pp., 1952 (December).
5 Pacific Division, Los Angeles, California, U. S. A., 20-21 June.
1953 19 Lawrence, Kansas, U. S. A., 25-27 June.
AMU Annual Report for 1953: 51 pp., 1953 (31 December).
6 Pacific Division, Pacific Grove, California, U. S. A., 12-13 June.
1954 20 Durham, New Hampshire, U. S. A., 16-18 August.
AMU Annual Report for 1954: 44 pp., 1954 (December).
7 Pacific Division, Los Angeles, California, U. S. A., 18-19 June.
1955 21 New York, New York, U. S. A., 26-29 July.
AMU Annual Reports for 1955 (Bulletin 22): 58 pp., 1955 (31 December).
8 Pacific Division, Stanford, California, U. S. A., 15-16 July.
1956 22 San Diego, California, U. S. A., 11-14 July.

AMU Annual Reports for 1956 (Bulletin 23): 52 pp., 1956 (31 December).
9 Pacific Division, Joint Meeting.
1957 23 New Haven, Connecticut, U. S. A., 16-19 July.
AMU Annual Reports for 1957 (Bulletin 24): 56 pp., 1958 (1 January).
10 Pacific Division, Santa Barbara, California, U. S. A., 30 May - 1 June.
1958 24 Ann Arbor, Michigan, U. S. A., 2-6 September.
AMU Annual Reports for 1958 (Bulletin 25): 73 pp., 1959 (1 January).
11 Pacific Division, Berkeley, California, U. S. A., 27-29 June.
1959 25 Philadelphia, Pennsylvania, U. S .A., 31 June - 3 July.
AMU Annual Reports for 1959 (Bulletin 26): 79 pp., 1960 (1 January) [mislabeled as "26th" Meeting].
12 Pacific Division, Redlands, California, U. S. A., 9-12 July.
1960 26 Montreal, Quebec, Canada, 9-12 August.
AMU Annual Reports for 1960. (Bulletin 27): 76 pp., 1961 (1 January) [mislabeled as "27th" Meeting].
13 Pacific Division, Pacific Grove, California, U. S. A., 22-25 June.
1961 27 Washington, D. C., U. S. A., 19-23 June.
AMU Annual Reports for 1961 (Bulletin 28): 81 pp., 1961 (1 December) [mislabeled as "28th" Meeting].
14 Pacific Division, Goleta, California, U. S. A., 28 June-1 July.
1962 28 St. Petersburg, Florida, U. S. A., 31 July - 3 August.
AMU Annual Reports for 1961 (Bulletin 29): 68 pp., 1962 (1 December) [mislabeled as "29th" Meeting].
15 Pacific Division, Pacific Grove, California, U. S. A., 27-30 June.
1963 29 Buffalo, New York, U. S. A., 18-21 June.
AMU Annual Reports for 1963 (Bulletin 30): 83 pp., 1963 (1 December).
16 Pacific Division, Goleta, California, U. S. A., 26-29 June.
1964 30 New Orleans, Louisiana, U. S. A., 21-24 July.
AMU Annual Reports for 1964 (Bulletin 31): 102 pp., 1964 (1 December).
17 Pacific Division, Pacific Grove, California, U. S. A., 18-21 June.
1965 31 New York, New York, U. S. A., 20-23 July.
AMU Annual Reports for 1965 (Bulletin 32): 122 pp., 1965 (1 December).
18 Pacific Division, San Diego, California, U. S. A., 24-27 June.
1966 32 Chapel Hill, North Carolina, U. S. A., 22-27 August.
AMU Annual Reports for 1966 (Bulletin 33): 120 pp., 1966 (1 December).
19 Pacific Division, Seattle, Washington, U. S. A., 19-22 June.
1967 33 Ottawa, Ontario, Canada, 31 July - 6 August.
AMU Annual Reports for 1967 (Bulletin 34): 119 pp., 1968 (20 March).
20 Pacific Division, Pacific Grove, California, U. S. A., 28 June-1 July.
1968 34 Corpus Christi, Texas, U. S. A., 15-19 July.
AMU Annual Reports for 1968 (Bulletin 35): 97 pp., 1968 (27 December).
21 Pacific Division, Pacific Grove, California, U. S. A., 19-22 June [concurrent with WSM].
1969 35 Marinette, Wisconsin, U. S. A., 21-23 July.
AMU Annual Reports for 1969 (Bulletin 36): 96 pp., 1969 (19 December).
Rare and Endangered Mollusks of North America, Arthur H. Clarke, organizer.
Malacologia 10(1):1-56, 1970 (14 November).
1970 36 Key West, Florida, U. S. A., 16-20 July.
AMU Annual Reports for 1970 (Bulletin 37): 112 pp., 1971 (18 February).
Commercial Marine Mollusks of the United States, Arthur S. Merrill, organizer.
AMU Annual Reports for 1970:9-40.
Introduced Mollusks, Alan Solem, organizer.
AMU Annual Reports for 1970:52-56.
1971 37 Cocoa Beach, Florida, U. S. A., 15-19 July.
AMU Bulletin for 1971: 70 pp., 1972 (February).
Molluscan Aquaculture, William N. Shaw, organizer.
AMU Bulletin for 1971:12-27.
1972 38 Galveston, Texas, U. S. A., 9-14 July.
AMU Bulletin for 1972: 64 pp., 1973 (23 March).
Genetics, Cytogenetics and Hybridization, John B. Burch, organizer.
AMU Bulletin for 1972:34-40 [abstracts].
Malacological Review 6(2):139-203, 1974 (29 January).
1973 39 Newark and Greenville, Delaware, U. S. A., 24-28 June.
AMU Bulletin for 1973: 69 pp., 1974 (22 May).
1974 40 Springfield, Massachusetts, U. S. A., 3-7

August.
AMU Bulletin for 1974: 93 pp., 1975 (May).

1975 41 San Diego, California, U. S. A., 22-26 June.
AMU Bulletin for 1975: 94 pp., 1976 (30 January).
Eastern Pacific-Western Atlantic Faunal Affinities, Emily H. Vokes, organizer.
AMU Bulletin for 1975:44-54.

1976 42 Columbus, Ohio, U. S. A., 2-6 August.
AMU Bulletin for 1976: 89 pp., 1976 (30 December).
Current Trends in Malacology, Dorothea S. Franzen, organizer.
AMU Bulletin for 1976:54-67.

1977 43 Naples, Florida, U. S. A., 10-15 July.
AMU Bulletin for 1977: 118 pp., 1978?
Evolution and Adaptive Radiation of Mollusca, George M. Davis, organizer.
AMU Bulletin for 1977:92-97.

1978 44 Wilmington, North Carolina, U. S. A., 16-21 July.
AMU Bulletin for 1978: 86 pp., 1979?
The Hows, Whys, and Wherefores of Building a Scientifically Valuable Mollusk Colllection, Carol B. Stein, organizer.

1979 45 Corpus Christi, Texas, U. S. A., 5-11 August.
AMU Bulletin for 1979: 86 pp., 1980 (March).
Mollusks of the Gulf of Mexico, Thomas E. Pulley, organizer.
AMU Bulletin for 1979:70-71 [abstracts].
Life Histories of Mollusks, David R. Lindberg and Michael G. Kellogg, organizers [see under WSM].

1980 46 Louisville, Kentucky, U. S. A., 19-25 July.
AMU Bulletin for 1980: 94 pp., 1981.
Functional Morphology of Cephalopoda, William Hulet, organizer.
Malacologia 23(1):87-208, 1982 (18 August).
Feeding Mechanisms of Predatory Mollusks, Alan Kohn, organizer.
Malacologia 20(2):359-469, 1981 (17 June).

1981 47 Fort Lauderdale, Florida, U. S. A., 19-25 July.
AMU Bulletin for 1981: 76 pp., 1982.
Morphology, Ontogeny and Higher Category Systematics of Molluscs, Richard S. Houbrick, organizer.
AMU Bulletin for 1982: 4 unpaginated pages of abstracts, 1983.
Malacologia 25(1): 264 pp., 1984 (29 March).
Second AMU Symposium on Endangered Mollusks of North America, Arthur H. Clarke, organizer [first: see 1969].
AMU Bulletin for 1981:41-59, 1982.

1982 48 New Orleans, Louisiana, U. S. A., 19-23 July.
American Malacological Bulletin 1:81-132, 1983.
Shell Microstructure, Robert S. Prezant, organizer.
American Malacological Bulletin 1:101-102 [abstracts].
Second International Symposium on Molluscan Genetics, George M. Davis, organizer [first: see listing at end, 1980].
American Malacological Bulletin 1:103-110 [abstracts].
Malacologia 25(2):265-648, 1984 (29 August).

1983 49 Seattle, Washington, U. S. A., 7-13 August.
American Malacological Bulletin 2:77-125, 1984.
Molluscan Nervous System and Behavior, A. O. Dennis Willows, organizer.
American Malacological Bulletin 2:78 [abstracts].
Malacologia 27(1):1-81, 1986 (7 March).
Molluscan Extinctions in the Geologic Past and [at] the Present Time, Geerat J. Vermeij, organizer.
American Malacological Bulletin 2:79-80 [abstracts].
Avian Molluscivores, David R. Lindberg, organizer.
American Malacological Bulletin 2:80 [abstracts].
Support Services in Malacology, Clyde F. E. Roper, organizer.
American Malacological Bulletin 2:80-81 [abstracts].

1984 50 Norfolk, Virginia, U. S. A., 22-27 July.
American Malacological Bulletin 3(1):91-130, 1985.
Physiological Ecology of Freshwater Molluscs Honoring Dr. W. D. Russell-Hunter, Albert J. Burky and Robert F. McMahon, organizers.
American Malacological Bulletin 3(2):135-272, 1985 (June).
Ecology of Larval Molluscs, Michael Vecchione, organizer.

American Malacological Bulletin 4(1):43-104 [mixed papers, abstracts], 1986.

1985 51 Kingston, Rhode Island, U. S. A., 28 July - 2 August.

American Malacological Bulletin 4(2): 230-244, 1986.

Ecology of Freshwater Molluscs, Eileen H. Jokinen, organizer.

American Malacological Bulletin 5(1): 1-128, 1987.

Encapsulation of Embryos, Jan A. Pechenik, organizer.

American Malacological Bulletin 4(2): 165-229, 1986.

1986 52 Monterey, California, U. S. A., 1-6 July.

American Malacological Bulletin 4(2): 230-224, 1986 [abstracts].

American Malacological Bulletin 5(1): 129-149, 1987 [meeting incorrectly listed as August].

Life History, Systematics and Zoogeography of Cephalopods in Honor of S. Stillman Berry, Roger Hanlon, organizer.

American Malacological Bulletin 4(2): 239-242 [abstracts], 1986.

Malacologia 29(1): 307 pp. [symposium date incorrectly stated as 1987], Roger T. Hanlon, ed., 1988 (28 June).

Molluscan Morphometric Analysis, David R. Lindberg and Carole S. Hickman, organizers.

American Malacological Bulletin 4(2): 242-244, 1986 [abstracts].

The Biology of Opisthobranch Mollusks, Honoring Dr. Eveline du Bois Reymond Marcus, Terrence M. Gosliner and Michael T. Ghiselin, organizers.

American Malacological Bulletin 5(2): 181-306, 1987.

1987 53 Key West, Florida, U. S. A., 19-23 July.

Biology of the Polyplacophora, Robert C. Bullock, organizer.

American Malacological Bulletin 6(1):55-159, 1988.

Cenozoic Fossil Molluscan Communities, Emily Vokes and Lyle Campbell, organizers.

1988 54 Charleston, South Carolina, U. S. A., 19-25 June.

Applications of Nucleic Acid Techniques to the Study of Molluscan Evolution, M. G. Harasewych, organizer.

History of Malacology, Wim Backhuys, organizer.

Systematics and Evolution of Nonmarine Mollusks, Robert Hershler, organizer.

1989 55 Los Angeles, California, U. S. A., 25-30 June.

Biology of Pelagic Gastropods, Roger P. Seapy, organizer.

American Malacological Bulletin 8(1): 25-59, 1990.

Biology of Scaphopods, Ronald L. Shimek, organizer.

Systematics, Anatomy and Evolution of Western North American Land Mollusks, in Honor of Walter B. Miller, Fred G. Hochberg and Barry Roth, organizers.

American Malacological Bulletin 8(2): 145-175, 1991.

1990 56 Wood Hole, Massachusetts, U. S. A., 3-8 June.

Integrative Neurobiology and Behavior of Mollusks, Roger T. Hanlon and Alan M. Kuzirian, organizers.

American Malacological Bulletin 9(1): 43-98, 1991.

Systematics, Biology and Fisheries of Recent Cephalopods in Honor of Gilbert L. Voss, Clyde F. E. Roper, Michael Vecchione, and Michael J. Sweeney, organizers.

Gilbert L. Voss International Symposium held at the American Malacological Union Annual Meeting at Marine Biological Biological Laboratory, Woods Hole, Massachusetts, June 3-7, 1990: contributions from friends, colleagues and students. *Bulletin of Marine Science* 49(1-2, Gilbert L. Voss Memorial Issue): 670 pp. [including non-symposium papers], 1991 (September).

1991 57 Berkeley, California, U. S. A., 30 June - 5 July.

Marine Bivalve Research in the Next Century: a Review of the Current State of our Knowledge and Directions for the Future, Paul H. Scott, Eugene V. Coan, and Brian Morton, organizers.

American Malacological Bulletin 9(2):107-215, 1992.

Molluscan Taphonomy and Paleoecology, Carole S. Hickman and Michael P. Russell, organizers.

History of the North Pacific Molluscan Fauna, David R. Lindberg and Geerat J. Vermeij, organizers.

1992 58 Sarasota, Florida, U. S. A., 2-7 August.
Biology of Caribbean Mollusks, Rüdiger Bieler, organizer.
American Malacological Bulletin 10(1):179-290, 1993.
Phylogeny and Classification of Gastropods, Terrence M. Gosliner, organizer.
1993 59 *Nordic Empress*, out of Miami, Florida, U. S. A., 21-25 June.
Patterns of Speciation, Kenneth C. Emberton and Robert Hershler, organizers.
1994 60 Houston, Texas, U. S. A., 9-14 July.
Gulf of Mexico Mollusca, Joseph C. Britton and John W. Tunnell, Jr., organizers.
Unionid workshop, Bob Howells, organizer.
1995 61 Hilo, Hawaii, U. S. A., 7-13 July.
Island Biogeography, Gustav Paulay, organizer.
American Malacological Bulletin 12(1/2):3-75, 1996.
Evolution of Coleoid Cephalopods, Richard E. Young, organizer.
American Malacological Bulletin 12(1/2):79-151, 1996.
Conservation, K. Elaine Hoagland, organizer.

Western Society of Malacologists [for 1968 and prior, see AMU, Pacific Division].
1968 1 Pacific Grove, California, U. S. A., 19-22 June.
Western Society of Malacologists Echo 1: 39 pp., 1969 (20 March).
Biology of Tomales Bay in Relation to the Environment, Edmund H. Smith, organizer.
1969 2 Pacific Grove, California, U. S. A., 18-21 June.
Western Society of Malacologists Echo 2: 84 pp., 1970 (9 March).
Scientific and Popular Publication in Malacology, Albert R. Mead, organizer.
Western Society of Malacologists Echo 2:27-31.
Nearshore Collecting Localities in the Gulf of California, Beatrice L. Burch, organizer.
Western Society of Malacologists Echo 2:31-39.
1970 3 Stanford, California, U. S. A., 24-27 June.
Western Society of Malacologists Echo 3: 77 pp., 1971 (7 March).
Nudibranchs, David R. Franz and Richard A. Roller, organizers.
Western Society of Malacologists Echo 3 [abstracts].
Advances in Molluscan Systematics: a Survey of New Theory and Practice, Eugene V. Coan, organizer.
Western Society of Malacologists Echo 3, 1971 [abstracts, some papers].
1971 4 Pacific Grove, California, U. S. A., 16-19 June.
Western Society of Malacologists Echo 4: 86 pp., 1971 (27 December).
Evolution of Muricacean Gastropods in Time and Space, William K. Emerson, organizer.
Western Society of Malacologists Echo 4:34-67.
Opisthobranchs, Steven J. Long, organizer.
Western Society of Malacologists Echo 4 [abstracts].
1972 5 Redlands, California, U. S. A., 18-21 June.
Western Society of Malacologists Echo 5: 101 pp., 1973 (5 March).
Ecology, James W. Nybakken, organizer.
Western Society of Malacologists Echo 5 [abstracts].
Opisthobranchs, Wesley M. Farmer, organizer.
Western Society of Malacologists Echo 5 [abstracts].
1973 6 Pacific Grove, California, U. S. A., 11-14 June.
Western Society of Malacologists Echo 6: 84 pp., 1974 (3 April).
Opisthobranchs, Gordon A. Robilliard, organizer.
Western Society of Malacologists Echo 6 [abstracts].
1974 7 Pomona, California, U. S. A., 19-22 June.
Western Society of Malacologists Annual Report 7: 73 pp., 1974 (12 November).
Paleontology, Louie N. Marincovich, Jr., organizer.
Western Society of Malacologists Annual Report 7 [abstracts].
Opisthobranchs, Hans Bertsch, organizer.
Western Society of Malacologists Annual Report 7 [abstracts].
1975 8 San Diego, California, U. S. A., 22-26 June.
Western Society of Malacologists Annual Report 8: 37 pp., 1975 (1 November).
Eastern Pacific-Western Atlantic Faunal Affinities, Emily H. Vokes, organizer [see under AMU].
1976 9 Pacific Grove, California, U. S. A., 23-26 June.
Western Society of Malacologists Annual

Report 9: 71 pp., 1976 (12 October).

Limpets, James T. Carlton, organizer.

Western Society of Malacologists Annual Report 9:13-35.

1977 10 Pomona, California, U. S. A., 15-18 June.

Western Society of Malacologists Annual Report 10: 48 pp., 1977 (14 December).

1978 11 Santa Clara, California, U. S. A., 28 June - 1 July.

Western Society of Malacologists Annual Report 11: 43 pp., 1979 (9 January).

Pulmonata.

Western Society of Malacologists Annual Report 11:15-25 [also abstracts].

1979 12 Corpus Christi, Texas, U. S. A., 5-11 August.

Western Society of Malacologists Annual Report 12: 21 pp., 1980 (24 March) [see under AMU].

Life Histories of Mollusks, David R. Lindberg and Michael G. Kellogg, organizers.

Western Society of Malacologists Annual Report 12:8-12.

1980 13 Davis, California, U. S. A., 22-25 June.

Western Society of Malacologists Annual Report 13: 28 pp., 1981 (29 June).

1981 14 San Diego, California, U. S. A., 23-26 June.

Western Society of Malacologists Annual Report 14: 32 pp., 1982 (13 July).

1982 15 Redlands, California, U. S. A., 20-23 June.

Western Society of Malacologists Annual Report 15: 26 pp., 1983 (30 August).

Update on Panamic Mollusca.

Western Society of Malacologists Annual Report 15:10-13 [abstracts].

1983 16 Seattle, Washington, U. S. A., 7-13 August.

Western Society of Malacologists Annual Report 16: 56 pp., 1984 (31 May) [see under AMU].

Molluscan Nervous System and Behavior, A. O. Dennis Willows, organizer.

Support Services in Malacology, Clyde F. E. Roper, organizer.

Molluscan Extinctions in the Geologic Past and at the Present Time, Geerat J. Vermeij, organizer.

Avian Molluscivores, David R. Lindberg, organizer.

1984 17 Santa Cruz, California, U. S. A., 16-19 August.

Western Society of Malacologists Annual Report 17: 48 pp., 1985 (28 August).

Mollusk Fauna of Northwestern Baja California, Hans Bertsch, organizer.

Opisthobranchs, Terrence M. Gosliner, organizer.

Paleontology, George L. Kennedy, organizer.

Western Society of Malacologists Annual Report 17 [abstracts for all three].

1985 18 Santa Barbara, California, U. S. A., 18-21 August.

Western Society of Malacologists Annual Report 18: 47 pp., 1986 (31 January).

Hawaiian Mollusks, Beatrice L. Burch, organizer.

Western Society of Malacologists Annual Report 18 [abstracts].

1986 19 Monterey, California, U. S. A., 1-6 July.

Western Society of Malacologists Annual Report 19: 40 pp., 1987 (9 March) [see under AMU].

The Biology of Opisthobranch Mollusks, Honoring Dr. Eveline du Bois Reymond Marcus, Terrence M. Gosliner, and Michael T. Ghiselin, organizers.

Western Society of Malacologists Annual Report 19:18-24 [abstracts].

Life History, Systematics and [Zoogeography] of Cephalopods in Honor of S. Stillman Berry, Roger Hanlon, organizer.

Western Society of Malacologists Annual Report 19: 25 [abstracts].

Molluscan Morphometric Analysis, David R. Lindberg and Carole S. Hickman, organizers.

Western Society of Malacologists Annual Report 19: 26 [abstracts].

[for all three, see also under AMU].

1987 20 San Diego, California, U. S. A., 21-25 June.

Western Society of Malacologists Annual Report 20: 42 pp., 1988 (8 March).

Northern Gulf of California: its Molluscan Distributions, Physical Oceanography and Geologic History of the Past 10-12 Million Years, Judith Terry Smith, organizer.

Western Society of Malacologists Annual Report 20:5-19.

Molluscan Aquaculture, David L. Leighton, organizer.

Western Society of Malacologists Annual Report 20:20-25.

1988 21 Rohnert Park, California, U. S. A., 17-21 July.

Western Society of Malacologists Annual Report 21: 26 pp., 1989 (7 March).

Biogeography and Evolution of the Molluscan Fauna of the Galápagos Islands, Matthew J. James, organizer.

Western Society of Malacologists Annual Report 21:5-15.

Matthew J. James, ed., 1991, *Galápagos Marine Invertebrates: Taxonomy, Biogeography, and Evolution in Darwin's Islands,* Plenum, New York, xiv + 474 pp.

Molluscan Herbivore - Plant Interactions, Cynthia D. Trowbridge, organizer.

Western Society of Malacologists Annual Report 21:15-17.

1989 22 Los Angeles, California, U. S. A., 25-30 June.

Western Society of Malacologists Annual Report 22: 41 pp., 1990 (11 June) [see under AMU].

Biology of Pelagic Gastropods, Roger P. Seapy, organizer.

Western Society of Malacologists Annual Report 22:1-3.

Biology of Scaphopods, Ronald L. Shimek, organizer.

Western Society of Malacologists Annual Report 22:3-4.

Systematics, Anatomy and Evolution of Western North American Land Molluscs in Honor of Walter B. Miller, Fred G. Hochberg, and Barry Roth, organizers.

Western Society of Malacologists Annual Report 22:4-8.

1990 23 Seattle, Washington, U. S. A., 18-22 June.

Western Society of Malacologists Annual Report 23: 27 pp., 1991 (4 May).

Current Directions in Alaskan Malacology, Paul H. Scott and Nora R. Foster, organizers.

Western Society of Malacologists Annual Report 23:1-16.

1991 24 Berkeley, California, U. S. A., 30 June - 5 July.

Western Society of Malacologists Annual Report 24: 44 pp., 1992 (8 June) [see under AMU].

Marine Bivalve Research in the Next Century: a Review of the Current State of Our Knowledge and Directions for the Future, Paul H. Scott, Eugene V. Coan, and Brian Morton, organizers.

Western Society of Malacologists Annual Report 24:1-14 [abstracts].

Molluscan Taphonomy and Paleoecology, Carole S. Hickman and Michael P. Russell, organizers.

Western Society of Malacologists Annual Report 24:14-19 [abstracts].

History of the North Pacific Molluscan Fauna, David R. Lindberg and Geerat J. Vermeij, organizers.

Western Society of Malacologists Annual Report 24:19-23 [abstracts].

1992 25 Pacific Grove, California, U. S. A., 30 June - 3 July.

Western Society of Malacologists Annual Report 25: 36 pp., 1993 (12 February).

Cocos Island, Donald R. Shasky, organizer.

Western Society of Malacologists Annual Report 25:1-5.

Opisthobranchs, Terrence M. Gosliner, organizer.

Western Society of Malacologists Annual Report 25:14-25.

1993 26 La Jolla, California, U. S. A., 27 June - 1 July 1993.

Western Society of Malacologists Annual Report 26: 24 pp., 1994 (26 June).

Contemporary Research on Mollusca, Paul H. Scott and Douglas J. Eernisse, organizers.

Western Society of Malacologists Annual Report 26:1-4 [abstracts].

Malacofauna of Western Mexico, Hans Bertsch, organizer.

Western Society of Malacologists Annual Report 26:4-11 [abstracts].

1994 27 Santa Barbara, California, U. S. A., 26-30 June.

Western Society of Malacologists Annual Report 27: 27 pp., 1995 (31 October).

Systematics of Micromollusks, James H. McLean, organizer.

Western Society of Malacologists Annual Report 27:1-2 [abstracts].

Current Topics in the Biogeography of Mollusks, Terrence Gosliner and Henry Chaney, organizers.

Western Society of Malacologists Annual Report 27:3-8 [abstracts].

1995 28 Chena Hot Springs, Alaska, U. S. A., 2-5 June.

Western Society of Malacologists Annual Report 28: 20 pp., 1996 (15 February).

African Symposia

1970 1 Meeting of Malacologists Specializing in African Non-Marine Mollusca, Tervuren, Belgium, 26-27 November.

Achatina 1:3-4, A. C. van Bruggen, ed., 1970 [abstracts].

1973 2 Second Réunion des Malacologistes Spécialisés dans la Faune Africaine, Tervuren, Belgium, 11-12 January.
Achatina 4:59-60, P. L. G. Benoit, ed., 1973 [abstracts].

Australasian Scallop Workshop

1988 1 Taroona, Tasmania, Australia, July, M. Dredge, W. Zacharin, and L. Joll, organizers.
1993 2 Triabunna, Tasmania, Australia, 23-25 March, M. Dredge, W. Zacharin, and L. Joll, organizers.
Queensland Museum, Memoirs 36(2):239-376, 1993 (10 August).

Azores Workshops

1988 1 The Marine Fauna and Flora of the Azores, Workshop on Malacology, São Miguel, Azores, Portugal, 11-24 July.
A. M. Frias Martins, ed., 1990 (October), Proceedings of the First International Workshop of Malacology and Marine Biology, *Açoreana*, supplement: 173 pp.
1991 2 The Marine Fauna and Flora of the Azores, Workshop on Malacology, São Miguel, Azores, Portugal, 21 July - 3 August.
A. M. Frias Martins, ed., 1995 (May), Proceedings of the Second International Workshop of Malacology and Marine Biology, *Açoreana*, supplement: iv + 316 pp.

Babosa del Frijol [*Vaginulus* (Veronicellidae)]

1984 1 Primer Seminario Regional Sobre la Babosa del Frijol, April.
Keith L. Andrews, H. Barletta, and George E. Pilz, eds., 1984, *Memoria, Ceiba* (Tegucigalpa, Honduras) 26(1):55-112.
1985 2 II Seminario Centroamericano Sobre la Babosa del Frijol, 22-25 April.
Keith L. Andrews and George E. Pilz, eds., 1985, *Memoria, Ceiba* 28(2):145-320.

Congresses on Medical and Applied Malacology

1987 1 Monterrey, Nuevo Leon, Mexico, 2-6 June.
Journal of Medical and Applied Malacology, supplement 1: iv + 76 pp., 1990 [proceedings].
1990 2 Yonsei University, Seoul, South Korea, 25-28 July.
Journal of Medical and Applied Malacology 3:143-174, 1991 [proceedings].
1993 3 Camden, New South Wales, Australia, 18-22 October.

***Dreissena* Symposia**

(1) International Zebra Mussel Research Symposia

1990 1 Columbus, Ohio, U. S. A., 5-7 December, Ohio Sea Grant, F. Snyder.
Zebra Mussel Update 6:3-4, 1991 (31 January) [report].
Journal of Shellfish Research 10(1):243-260, 1991 (June) [abstracts].
1991 2 Rochester, New York, U. S. A., 19-22 November, Great Lakes Sea Grant Network (U. S. A.) and Fisheries and Oceans Canada.
Mussel Morsels 2(1):1-4, 1992 (17 January) [report].
Journal of Shellfish Research 11(1):211-241, 1992 (June) [abstracts].

(2) International Zebra Mussel Conferences

1991 1 Zebra Mussels: Mitigation and Options for Industries, Toronto, Ontario, Canada, 11-12 February.
Mussel Morsels 1(3):1, 1991 (1 March) [report].
Dresisena polymorpha [(Pallas, 1771)] *Information Review* 2(2):4-5, 1991 (April) [report].
Dreissena polymorpha *Information Review*, 1992 (July).
1992 2 Toronto, Ontario, Canada, 19-21 February.
Mussel Morsels 2(2):1-2, 27 March; 2(3): 1-4, 1992 (10 April) [report].
Dreissena polymorpha *Information Review*, 1992 (July).
1993 3 Toronto, Ontario, Canada, 23-26 February.
Zebra Mussel Update 16:2-3, 1993 (23 March) [report].
1994 4 Madison, Wisconsin, U. S. A., 7-10 March.

(3) Other *Dreissena* Symposia

1990 Zebra Mussel Symposium, North American Benthological Society, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, U. S. A., 22-25 May.
1990 Exotic Bivalves, American Society of Limnology and Oceanography, College of William and Mary, Williamsburg, Virginia, U. S. A., 12 June.
1990 Zebra Mussel Threat to Wisconsin, Milwaukee, Manitowoc, and Ashland, Wisconsin, U. S. A., Wisconsin Coastal Management Program, 28-29 August.

Zebra Mussel Update 4:3, 1990 (17 September) [report].

1990 Zebra Mussel Symposium, Detroit, Michigan, U. S. A., 12 December; Detroit Water and Sewerage Department, J. I. Williams.

1991 The Zebra Mussel, Zoologisches Institut der Universität Köln, Germany, 3-4 January.
D. Neumann and H. A. Jenner, eds., 1992, The zebra mussel *Dreissena polymorpha.* Ecology, biological monitoring and first applications in the water quality management, *Limnologie Aktuel* 4: viii + 262 pp., 1992 [also published as a book, Stuttgart, G. Fischer, x + 263 pp.].

1991 Zebra Mussel Workshop, Toronto, Ontario, Canada, 22-23 January; Ontario Ministry of Natural Resources, C. Brousseau.
Mussel Morsels 1(2):1-2, 1991 (1 February) [report].

1991 Zebra Mussels in the Inland Waterways: What You Should Know, Fort Mitchell, Kentucky, U. S. A., 11-12 June; Ohio River Basin Commission and Upper Mississippi River Basin Commission.

1991 Zebra Mussels. American Society of Limnology and Oceanography, Halifax, Nova Scotia, Canada, 10-13 June; A. Longhurst.

1991 Zebra Mussel Control Technology Conference, Itasca, Illinois, U. S. A., 22-23 October; Electric Power Research Institute Papers presented at the Electric Utility Zebra Mussel Research Conference, October 1991. Special Conference Issue, June-July 1992, *Dreissena polymorpha Information Review.*

1992 Zebra Mussels in the Upper Mississippi River System, Bloomington, Minnesota, U. S. A., 2-3 April; Upper Mississippi River Basin Association.

1994 Zebra Mussels in Michigan: Implications for Industry and Municipalities, Michigan State University, Lansing, Michigan, U. S. A., 2-3 April, C. Pistis ["Fourth Annual Meeting"].

1994 Third Annual Meeting on the Biology, Ecology and Control of Zebra Mussels at Public Facilities, Denver, Colorado, U. S. A., 10-12 May; L. Sanders.

1994 Invasion of Zebra Mussels in Lakes and Estuaries, Windsor, Ontario, Canada, 5-9 June; International Association for Great Lakes Research, D. Wright and D. MacNeill.

1995 The Biology, Ecology, and Physiology of Zebra Mussels, St. Louis, Missouri, U. S. A., 4-8 January; American Society of Zoologists, Robert McMahon and Jeffrey Ram.
American Zoologist 36(1):239-384, 1996.

Franco-British Symposia on Molluscs

1978 1 Brest, France, 26-28 September.
Haliotis 9(2): 136 pp., 1979 [as "1978"].

1982 2 London, England, United Kingdom, 6-9 September.
Journal of Molluscan Studies, supplement 12A: 227 pp., 1983.

International Abalone Symposia

1989 1 La Paz, Baja California Sur, Mexico, 21-25 November.
Programa y Resumenes, Centro Interdisciplinario de Ciencias Marinas, Instituto Politecnico Nacional La Paz, 107 pp.
S. A. Shepherd, M. J. Tegner, and S. A. Guzmán del Proo, eds., 1992, *Abalone of the World: Biology, Fisheries and Culture,* Fishing News Books, Oxford, xiv + 608 pp.
S. A. Guzmán del Proo, M. J. Tegner, and S. A. Shepherd, eds., 1992, Abalone of the world: biology, fisheries and culture: supplementary papers, South Australia, Department of Fisheries, *Fisheries Research Papers* 24:68 pp.

1994 2 Hobart, Tasmania, Australia, 7-11 February.
Australian Journal of Marine and Freshwater Research 46(3), 1995 [in press].

International *Corbicula* Symposia

1977 1 Fort Worth, Texas, U. S. A., 13-15 October, Joseph C. Britton, organizer.
Joseph C. Britton, ed., 1979, *Proceedings, First International Corbicula Symposium*, Texas Christian University Research Foundation, Fort Worth, 313 pp.

1983 2 Little Rock, Arkansas, U. S. A., 21-24 June, Louise Russert Kraemer, Robert West, Robert McMahon, Jack Mattice, Paul Hayes, and Joseph C. Britton, organizers.
Robert S. Prezant, ed., 1986 (June), Proceedings of the Second International *Corbicula* Symposium, *American Malacological Bulletin,* special edition 2: 239 pp.

European/International Meetings on Littorinid Biology
[see also Littorinidae Research Group]

1986 1 London, England, United Kingdom, 26 November.

1988 2 Tjärnö, Sweden, 4-8 July.

K. Johannesson, D. G. Raffaelli, and C. H. Ellis, eds., 1990 (29 March), Progress in littorinid and muricid biology, *Hydrobiologia* 193: xii + 285 pp.

1990 3 Dale Fort, Wales, United Kingdom, 5-12 September.

J. Grahame, P. J. Mill, and David G. Reid, eds., 1992, *Proceedings of the Third International Symposium on Littorinid Biology,* Malacological Society of London, vi + 324 pp.

1993 4 Roscoff, France, 19-25 September.

Hydrobiologia [in press].

P. Fiorini and D. Reid, eds., 1994, *Cahiers de Biologie Marine* 35(2):237-267 [abstracts].

International Chiton Symposia

1991 1 Adelaide, South Australia, Australia, 1-6 December.

Journal of the Malacological Society of Australia 13:1-72, 1992 (30 October).

International Pectinid Workshops

1976 1 Baltimore, Ireland.

1978 2 Brest, France.

1980 3 Port Erin, Isle of Man, United Kingdom.

1983 4 Aberdeen, Scotland, United Kingdom, May.

ICES Publications, CM 1983/K: 37, 20 pp.

1985 5 La Coruña, Galicia, Spain.

1987 6 Menai Bridge, Wales, United Kingdom.

1989 7 Portland, Maine, U. S. A.

S. E. Shumway, ed., 1991, *Scallops: Biology, Ecology and Aquaculture,* Elsevier, Amsterdam, xx + 1095 pp. *Developments in Aquaculture and Fisheries Science* 21 [partly an outgrowth of the symposium].

S. E. Shumway and P. A. Sandifer, eds., 1991, An international compendium of scallop biology and culture; a tribute to James Mason. Selected papers from the 7th International Pectinid Workshop sponsored by the National Shellfisheries Association, *World Aquaculture Workshops* 1: xxi + 357 pp.

1991 8 Cherbourg, France, 22-29 May.

Pierre Lubet, Jean Barret, and Jean-Claude Dao, eds., 1995, *Fisheries, Biology and Aquaculture of Pectinids,* IFREMER, Brest, France, 273 pp.

1993 9 Nanaimo, British Columbia, Canada, 22-27 April.

Neil F. Bourne, B. L Bunting, and L. D. Townsend, eds., 1994, Proceedings of the 9th International Pectinid Workshop, Nanaimo, B.C., Canada, April 22-27, 1993. *Canadian Technical Report of Fisheries and Aquatic Sciences* 1994, 1: xvi + 222 pp.; 2: xvi + 217 pp.

1995 10 Cork, Ireland, 26 April - 2 May.

Latin American Malacological Congresses

1991 1 Caracas, Venezuela, 15-18 July.

Resumenes, 210 pp.

Strombus, Richard S. Appeldoorn, organizer.

Resumenes:131-190 [abstracts].

Richard S. Appeldoorn and Bladimir Rodriguez Q., eds., 1994, *Biologia, Pesqueria y Cultivo de Caracol – Strombus gigas [Linné, 1758] – Queen Conch Biology, Fisheries and Mariculture,* Funcacion Cientifica Los Roques, Caracas, Venezuela, 356 + 2 pp.

1995 2 Porto Alegre, Brasil, 11-16 July 1995.

Programa e Resumos, iii + 131 pp.

Littorinidae Research Group

[see also European/International Littorinid Meetings]

1974 Taxonomic Trends in the Littorinidae, Edinburgh, Scotland, United Kingdom, 19 March.

The Littorinid Tidings 1:1-10, 1974 (April) [abstracts].

1974 Bangor, Wales, United Kingdom, 19-21 August.

The Littorinid Tidings 2:20-24, 1974 (September); 3:27-29, 1975 (March).

1975 Roscoff, France, 13 May.

The Littorinid Tidings 4:36-38, 1975 (October).

Malacological Society of London

[includes joint meetings with other organizations, but does not include its Seminars]

1959 The Status of the Protobranchia within the Bivalve Mollusca, London, England, United Kingdom, 27 February; J. E. Morton, Linnean Society of London.

Malacological Society of London, Proceedings 33(5):200-223, 1959 (November).

1967 Structure, Physiology and Ecology of Molluscs, London, Zoological Society of London and Malacological Society of London, 8-9 March.

V. Fretter, ed., 1968, Studies in the structure, physiology and ecology of molluscs, *Zoological Society of London, Symposia* 22: xviii + 377 pp.

1976 Symposium, Sea-Slugs and Land-Slugs, Edinburgh, Scotland, United Kingdom, 26-27 March, Royal Scottish Museum, Malacological Society of London, and Conchological Society of Great Britain and Ireland.
Journal of Molluscan Studies 42(2):295-302, 1976 (October) [abstracts].

1976 Malacological Society of London and Underwater Association of Research Scientists, Bristol, England, United Kingdom, September.
Journal of Molluscan Studies 42(3):451-456, 1976 (December) [abstracts].

1977 Evolutionary Systematics of Bivalve Molluscs, London, England, United Kingdom, 18-19 May.
C. M. Yonge and T. E. Thompson, eds., 1978 (16 November), Evolutionary systematics of bivalve mollusks, a discussion organized by the Royal Society and the Malacological Society of London, 18-19 May 1977, *Royal Society of London, Philosophical Transactions (B - Biological Sciences)* 284(1001):199-436.

1983 Molluscan Research in Scotland, Millport, Scotland, United Kingdom, 12-13 March.
University Marine Biological Station, Millport, Occasional Publication 1: 41 pp., 1984 (April).

1993 Malacological Society of London, Centenary Symposium, London, England, United Kingdom, 14-16 September.
John D. Taylor, ed., 1995 (November), *Origin and Evolutionary Radiation of the Mollusca*, Oxford University Press, 464 pp.

1993 Evolution and Systematics of Patellidae, Malacological Society of London, London, England, United Kingdom, 17 September.

1994 Malacological Pioneers, Malacological Society of London, Conchological Society of Great Britain and Ireland, Society for the History of Natural History, London, England, United Kingdom, 22 October.
The Conchologists' Newsletter 130:394-395, 1994 [abstracts].
The Conchologists' Newsletter 132:446-449, 1995 (March) [report].
Malacological Society of London, Bulletin 24:7-13, 1995 (February) [report, abstracts].
Archives of Natural History 22(3):301-435, 1995 (October).

1994 Speciation and Adaptive Radiation in Molluscs, Cambridge, England, United Kingdom, 8 November.
Malacological Society of London, Bulletin 24:3-6, 15, 1995 (February) [report, abstracts].

Polish National Malacological Seminars [Krajowe Seminarium Malakologiczne]

1985 1 Kroscienko nad Dunajcem, Poland, 15-18 April.
Przeglad Zoologiczny 29(4):541-542 [report by K. Lewandowski].

1986 2 Kroscienko nad Dunajcem, Poland.

1987 3 Kroscienko nad Dunajcem, Poland.

1988 4 Krajowe, Poland, 21-24 March.
Przeglad Zoologiczny 32(4):643-644 [report by K. Lewandowski].

1989 5 Krajowe, Poland, 27 February - 2 March.
Przeglad Zoologiczny 33(4):638-639 [report by K. Lewandowsk].

Reunion Nacional de Malacologia y Conquiliologia (Mexico)

1984 1 La Paz, Baja California Sur, Mexico, March.
Memorias, 97 pp., 1985?

1986 2 Villahermosa, Tabasco, Mexico.
Memorias, 380 pp., 1987?

1987 3 Monterrey, Nuevo Leon, Mexico.
Memorias, 546 pp., 1988?

1990 4 La Paz, Baja California Sur, Mexico, 9-12 October.
Resumenes, unpaginated, 1990.

1992 5 Morelia, Michoacan, Mexico, 1-5 December.
Resumenes, 54 pp., 1992.

Rudist Conferences

1988 1 Belgrade, Serbia [Yugoslavia], 24-26 October.
Serbian Geological Society, viii + 40 pp. [abstracts].

1990 2 Rome-Bari, Italy, 1-7 October.
Geologica Romana (n.s.) 28: xli + 372 pp. [proceedings].
[also, dedicatory volume published by Servizio Geologico Nazionale, 1990, 450 pp., reprinting papers by C. F. Parona on rudistids].

1993 3 Mexico City, Mexico, October.

Società Italiana di Malacologia

1984 1 Palermo, Italy, 13-15 September.

Società Italiana di Malacologia, Lavori 22: 364 pp., 1986 [proceedings].

1987 2 Sorrento, Italy, 29-31 May.
P. Crovato and G. F. Russo, eds., 1990 (November), *Società Italiana di Malacologia, Lavori* 23: 512 + vi pp. [proceedings].

1990 3 Parma, Italy, 11-13 October.
D. Bedulli, ed., 1993 (February), *Società Italiana di Malacologia, Lavori* 24: vi + 304 pp. [as "1992"] [proceedings].

1994 Palermo (Gibilmanna), Italy, 7-9 October, Workshop on marine molluscan communities in the past and in the present, R. Chemello *et al.*
Bollettino Malacologico [in press].

Société Française de Malacologie

1970 1 Caen, France, 7-12 September.
Haliotis 1(1):1-60; 1(2):61-232, 1971.

1973 2 Lyon, France, 4-8 September.
Haliotis 4(1-2): 216 pp., 1976 [as "1974"].

1975 3 La Rochelle, France, 16 June.
Haliotis 6:219-313, 1977 [as "1976"].

1978 4 Brest, France, 25 September.
Haliotis 9(1): 102 pp., 1979 [as "1978"].

1983 5 Ile des Embiez, Var, France, 5 September.
Haliotis 13: [iv] + xx + 168 pp., 1984 [as "1983"].

1985 6 Wimereux, France, 4-8 November [includes Colloque "Contamination, Intoxication et Perturbation des Mollusques Marins"].
Haliotis 15: [vi] + xx + 384 pp., 1986.

1987 7 Rennes, France, 31 August - 2 September.
Haliotis 18: xvi + 313 pp., 1988.

1990 8 Brest, France, 7-8 November, "Aspects Récents de la Biologie de Mollusques."
Haliotis 21: 194 pp., 1992 [as "1991"] [Actes de Colloques 13].

1993 9 La Rochelle, France, 7-10 July, "Malacologie et l'Environment."
Haliotis 23: 171 pp., 1994 [as "1993"].

Society for Experimental and Descriptive Malacology

1969 1 Ann Arbor, Michigan, U. S. A.
Malacological Review 2(2):131-132, 1970 (8 May).

1971 2 Ann Arbor, Michigan, U.S.A., 9 August.
Malacological Review 5(1):9-22, 1972 (31 July).

1972 3 Ann Arbor, Michigan, U. S. A., 15-26 August.
Malacological Review 6(1):41-68, 1973 (12 April).

1973 4 Ann Arbor, Michigan, U. S. A., 24-25 August.
Malacological Review 7(1):40-58, 1974 (30 September).

1974 5 Ann Arbor, Michigan, U. S. A., 22 June.
Malacological Review 8(1-2):116-128, 1975 (15 May).

Soviet/Russian Malacological Meetings

1961 1 [no information available; evidently no publications].

1965 2 Molluscs: Theoretical Questions and Applied Malacology, 8-12 February.
Leningrad, U. S. S. R., 101 pp., 1965 (post-1 February) [abstracts].

1968 3 Molluscs and their Role in Ecosystems.
Leningrad, U. S. S. R., 95 pp., 1968 (post-27 March) [abstracts].

1971 4 Molluscs: Trends, Methods and Some Results of the Investigations, March.
Leningrad, U. S. S. R., 158 pp., 1971 (post-11 February) [abstracts].

1975 5 Molluscs: Their System, Evolution and Significance in Nature, February.
Leningrad, U. S. S. R., 242 pp., 1975 [abstracts].
Translated: *Malacological Review* 11(1-2):63-156, 1978 (20 July).

1979 6 Molluscs: Main Results of their Study, 7-9 February.
Leningrad, U. S. S. R., 261 pp., 1979 (probably early in year) [abstracts].
Some abstracts translated: *Malacological Review* 17(1-2):105-149, 1984 (11 April).

1983 7 Molluscs: Their Systematics, Ecology and Distribution, 5-7 April.
Leningrad, U. S. S. R., 262 pp., 1983 (post-22 February) [abstracts].

1987 8 Molluscs: Results and Perspectives of Investigation, April.
Leningrad, U. S. S. R., 536 pp., 1987 (post-23 October) [abstracts].

1991 9 16-18 April [no additional information available].

Symposia on Molluscan Neurobiology [SYMON]

1982 1 Amsterdam, The Netherlands, 16-20 August.
J. Lever and H. H. Boer, eds., 1982, *Molluscan Neuroendocrinology*, Proceedings of the International Minisymposium on Molluscan Endrocrinology, North-Holland, Amsterdam, 268 pp.

1986 2 Amsterdam, The Netherlands, 18-22 August.
Comparative Biochemistry and Physiology 86A(4):785-786 [report].

H. H. Boer, W. P. M. Geraerts, and J. Joosse, eds., 1986, *Neurobiology Molluscan Models,* North-Holland, Amsterdam, 376 pp.

1990 3 Amsterdam, The Netherlands, 20-24 August.
K. S. Kits, H. H. Boer, and J. Joosse, eds., 1991, Molluscan neurobiology, *Koninklijke Nederlandse Akademie van Wetenschappen, Verhandelingen, Afdeling Natuurkunde* (ser. 2) 88: 360 pp.

1994 4 Amsterdam, The Netherlands, 1-4 June.
Karel S. Kits, Harry H. Boer, and August B. Smit, eds., 1995 (February), Proceedings of the Fourth Symposium on Molluscan Neurobiology, *Netherlands Journal of Zoology* 44(3/4): 598 pp.

Workshop Malakozoologie

1981 1 Münster, Westfalen, Germany, 9-11 October, Jürgen H. Jungbluth, organizer.
Mitteilungen der Deutschen Malakozoologischen Gesellschaft 3, supplement: 1-70, 1982 (April).

1983 2 Münster, Westfalen, Germany, 25-27 February, Evolution der Mollusken, Jürgen H. Jungbluth, organizer.
Mitteilungen der Deutschen Malakozoologischen Gesellschaft 37:1-236, 1984.

Workshops on the Malacofauna of Hong Kong and Southern China

1977 1 Hong Kong, 23 March - 8 April.
Brian Morton, ed., 1980, *Proceedings of the First International Workshop on the Malacofauna of Hong Kong and Southern China,* Hong Kong University Press, vi + 352 pp.

1983 2 Hong Kong, 6-24 April.
Brian Morton and C. K. Tseng, eds., 1985, *Proceedings of the Second International Workshop on the Malacofauna of Hong Kong and Southern China*, Hong Kong University Press, 2 vols., viii + 681 pp.

1992 3 Hong Kong, 13 April - 1 May.
Brian Morton, ed., 1994, *The Malacofauna of Hong Kong and Southern China III,* Hong Kong University Press, 524 pp.

Workshops of the Tropical Marine Mollusc Programme (TMMP)

1991 1 Phuket, Thailand, 12-18 August.
Proceedings of the First Workshop of the Tropical Marine Mollusc Programme, 1991, *Phuket Marine Biological Center, Special Publication* 9: 142 pp.

1992 2 Annamalai University, India, 4-14 May.
Proceedings of the Second Workshop of the Tropical Marine Mollusc Programme, 1992, *Phuket Marine Biological Center, Special Publication* 10: 240 pp.

1992 3 Phuket Marine Biological Center, Thailand, 1-4 November.
Proceedings of the Third Workshop of the Tropical Marine Mollusc Programme, 1992, *Phuket Marine Biological Center, Special Publication* 11: 178 pp.

1993 4 Phuket Marine Biological Center, Thailand, 27 October - 2 November.
Proceedings of the Fourth Workshop of the Tropical Marine Mollusc Programme, 1994, *Phuket Marine Biological Center, Special Publication* 13: iv + 246 pp.

1995 5 Sulawesi, Indonesia, September.
Proceedings of the Fifth Workshop of the Tropical Marine Mollusc Programme, *Phuket Marine Biological Center, Special Publication* [in press].

Other Symposia and Workshops

1968 Symposium on Molluscs, Cochin, India, 12-16 January, K. Virabhadra Rao, organizer.
Proceedings, Symposium Series 3, Marine Biological Society of India, Mandapam Camp, 1968-1970: 1: xxxv + 386 pp., 1968; 2: iv + 387-706 pp., 1969; 3: iv + 707-1052 pp., 1970.

1969 Materialy Mezhvuzovskoy Nauchno-Metodicheskoy Konferentsii po Izucheniyu Presnovodnikh Molliuskov Sibiri, 26-28 June, Tomsk, Siberia, U. S. S. R.
B. G. Ioganzen, ed., 1969, *Voprosy Malakologii Sibiri [Malacological Problems in Siberia]*, Tomsk, Izdatel'stvo Tomskogo Universiteta, 202 pp.
Ye. A. Novikov, ed., 1970, Interuniversity Conference on Methods for Studying Siberian Freshwater Mollusks, *Hydrobiological Journal* 6(1):118-119.

1971 Rare and Endangered Mollusks (Naiads) of the U. S., Columbus, Ohio, U. S. A., 6 April.
S. E. Jorgensen and R. W. Sharp, eds., 1971 (10 August), *Proceedings of a Symposium on Rare and Endangered Mollusks (Naiads) of the U. S.*, U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries

and Wildlife, Region 3, vi + 79 pp.

1971 1ére Reunion de la Société Française de Malacologie et de la Società Malacologica Italiana, Lyon, France, 1-3 May.
Haliotis 2(1): 48 pp., 1972.

1972 Le Calcium Chez les Mollusques, Besançan, France, 25-26 May.
Haliotis 2(2):49-219, 1974.

1972 Le Malacofaune Terziarie e Quaternarie: Loro Paleoecologia e Paleobiogeografia, Verona, Italy, 9-10 September, Società Malacologia Italiana.
Memorie del Museo Civico di Storia Naturale di Verona 20:567-589, 1974 [as "1972"].

1972 Premier Colloque de Malacologie Appliquée, Rouen, France, 28-30 September.
Haliotis 3(1-2): 208 pp., 1974 [as "1973"].

1974 Colloquium on Mollusca Phylogeny, London, England, United Kingdom, 3-4 April, International Palaeontological Association.
The Littorinid Tidings 2:10-12 [abstracts of two papers].

1975 Polymorphisme et Dimorphisme chez les Mollusques Fossiles et Actuels, Dijon, France, 7-8 March.
Haliotis 6: 320 pp., 1977 [as "1976"].

1975 Colloque International de Malacologie Marine Appliquée, La Rochelle, France, 16-20 June.
Haliotis 5: 300 pp., 1976 [as "1975"].

1975 Feeding in Gastropod Mollusks: Behavioral and Neurophysiological Substrates – A Symposium, Woods Hole, Massachusetts, U. S. A., 14 August.
A. Gelperin, ed., 1977 (1 July), *The Veliger* 20(1):55-61 [abstracts].

1975 Mollusca, with Special Reference to the Sydney Area, Sydney, New South Wales, Australia, 30-31 August, Winston F. Ponder, organizer.
Malacological Review 9(1-2):131-141, 1976 (26 March) [abstracts].

1976 Ecophysiologie des Mollusques Marins et d'Eaux Saumatres; Impact des Pollutions, Ile de Embiez, Var, France, 13-16 September.
Haliotis 7: 166 pp., 1978 [as "1976"].

1976 Papers on Neogene Mollusks of the North Pacific Margin, First International Congress on Pacific Neogene Stratigraphy, Tokyo, Japan, 17-21 May 1976.
Warren O. Addicott, ed., 1978 (1 October), Papers on Neogene Mollusks of the North Pacific Margin, *The Veliger* 21(2):153-235.

1977 Premier Colloque International de Pathologie et Parasitologie des Mollusques, Perpignan, France, 9-12 September 1977.
Haliotis 8: 346 pp., 1979.

1979 Biologie des Escargots – Exploitation, Production, Heliciculture, Auzeville, France, 10-12 September.
Haliotis 10(1): 100 pp., 1980.

1979 Symposium on the Biology and Evolution of Mollusca, Sydney, New South Wales, Australian Museum, 21-25 May, Winston F. Ponder and A. J. Underwood, organizers.
Journal of the Malacological Society of Australia 4(4):223-263, 1979 (30 June) [abstracts].

1980 First International Symposium on Molluscan Genetics, London, England, United Kingdom, 17-19 April.
J. S. Jones, 1980 (29 May), Evolutionary genetics of snails, *Nature* 285:283-285 [report] [second: see AMU, 1982].

1980 Molluscan Nerve Cells, Cold Spring Harbor, New York, U. S. A., 18-21 May.
J. Koester and J. H. Byrne, eds., 1980, *Cold Spring Harbor Reports in the Neurosciences* 1: xviii + 230 pp.

1981 Freshwater Mollusks Workshop, Vicksburg, Mississippi, U. S. A., 19-20 May.
A. C. Miller, ed., 1983 (October), U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, x + 184 pp. [report].

1981 First North American Symposium on *Littorina*; *Littorina* Minisymposium and Workshop ["Ecological effects and biogeogeography of an introduced marine species: the periwinkle, *Littorina littorea* [(Linné, 1758)] (a zoological, botanical and ecological symposium)"], Nahant, Massachusetts, U. S. A., 28-30 August, James T. Carlton, D. P. Cheney, and Geerat J. Vermeij, organizers.
Abstracts issued at meeting: 16 pp.
Malacological Review 15(1-2):143-150, 1982 (4 May) [abstracts].

1982 All-Union School Symposium on Gastropoda, Dushanbe, Tajikistan, U. S. S. R., 6-17 September.
Paleontologicheskii Zhurnal 1983(1):138-140, O. V. Amitrov and R. M. Dzhililov, 1983.

1982 Sistematica dei Prosobranchi del Mediterraneo, Bologna, Italy, 24-26 September.
Società Italiana di Malacologia, Lavori

21: 239 pp., 1985 [as "1984"].

1982 Freshwater Mussels Workshop, Vicksburg, Mississippi, U. S. A., 26-27 October.
A. C. Miller, ed., 1982, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 184 pp. [report].

1983 Conchyliculture Mediterraneenne, Ile des Embiez, Var, France, 8-9 September.
Haliotis 14: [iv] + xvi + 178 pp., 1984.

1984 First PMBC/Danida Training Course and Workshop on Taxonomy of Marine Bivalves, with Emphasis on Economic Species, Phuket, Thailand, 23-28 January.
Phuket Marine Biological Center, Special Publication 2: 12 pp., 1986 [paper by B. Phasuk and J. Hylleberg].

1985 Perspectives in Malacology; a Symposium to Honor M. R. Carriker, Newark, Delaware, U. S. A., 7 February, Franklin C. Daiber, organizer.
Robert S. Prezant and Clement L. Counts, III, eds., 1985 (July), Perspectives in malacology, *American Malacological Bulletin, Special Edition* 1: 116 pp.

1985 Entrainment of Larval Oysters by Cutterhead Dredges in Chesapeake Bay, University of Delaware, Lewes, U. S. A., August.
Robert S. Prezant, ed., 1986 (October), Entrainment of larval oysters, *American Malacological Bulletin, Special Edition* 3: 74 pp.

1985 Short Course on Mollusks, Paleontological Society, Orlando, Florida, U. S. A., 27 October.
D. J. Bottjer, C. S. Hickman, and P. D. Ward, eds., 1985, Mollusks; notes for a short course, *University of Tennessee, Studies in Geology* 13: iv + 305 pp.

1986 First Workshop on Molluscs, Cebu City, Philippines.
Carfel Philippine Shell News 8(3):5-6 [report by J. J. Cabrera].

1986 Workshop on Die-Offs of Freshwater Mussels in the United States, Davenport, Iowa, U. S. A., 23-25 June.
R. J. Neves, ed., 1987, U. S. Fish and Wildlife Services, Upper Mississippi River Conservation Committee, Rock Island, Illinois, U. S. A., 166 pp.

1986 Regional Training Course in Taxonomy, Biology, and Culture of Commercial Species of Molluscs, Phuket, Thailand, 5 February - 14 March.
Phuket Marine Biological Center, Special Publication 4: 22 pp., 1986 [paper by B. Phasuk and J. Hylleberg].

1986 Expert Consultation on Shared Fishery Resources of the Lesser Antilles Region, Mayaguez, Puerto Rico, 8-12 September [*Strombus gigas* fisheries].
R. Mahon, ed., 1987, Report and proceedings, *FAO Fisheries Report* 383: v + 278 pp.

1986 Ecologie, Ecophysiologie, Energétique des Mollusques Marins et Continentaux, Rochefort, France, 9-13 September.
Haliotis 16: xiv + 592 pp., 1987.

1987 Halifax Sea Scallop Workshop, 13-14 August, Halifax, Nova Scotia, Canada.
Canadian Technical Report of Fisheries and Aquatic Sciences 1605: v + 12 pp. [summary].

1987 Biologie Appliquée à la Conchyliculture et à l'Héliciculture, Rennes, France, 2-5 September.
Haliotis 19: [vi] + 384 pp., 1989.

1988 Second All-Union School Symposium on Molluscs, Dushanbe, Tajikistan, U. S. S. R., 19-29 March.
Paleontologicheskii Zhurnal 1989(1):36-137, 1989 [report by O. V. Amitrov].

1988 All-Union Colloquium on Bivalve and Gastropod Mollusks, Moscow, U. S. S. R., 24-27 March.
Paleontologicheskii Zhurnal 1989(2):125-126, 1989 [report by O. V. Amitrov].

1988 Giant Clams in Asia and the Pacific, James Cook University, Townsville, Queensland, Australia, April.
J. W. Copland and J. S. Lucas, eds., 1988, *Queensland Centre for International Agricultural Research (ACI Annual Report), Monograph* 9: 274 pp. A. C. T., Canberra, [proceedings].

1988 Australasian Scallop Workshop, Taroona, Tasmania, Australia, July.
M. L. C. Dredge, W. F. Zacharin, W. F. Sacharin, and L. M. Joll, eds., 1989 (December), Tasmanian Government Printer, Hobart, iv + 290 pp. [proceedings].

1988 Das *Mitra*-Minisymposium, Eberbach, Switzerland.
Société Internationale de Conchyliologie [Lausanne], Bulletin 10(4):8-11 [report by H. Turner].

1989 Snails 1989, Edinburgh, Scotland, United Kingdom, 4 March [*Helix pomatia* Linné, 1758, culture].

R. E. Groves, ed., 1989, The Snail Centre, Plas Newydd, Wales, 80 pp. [transcript].

1989 Slugs and Snails in World Agriculture, United Kingdom, April.

I. F. Henderson, ed., 1989 (April), Slugs and snails in world agriculture, *British Crop Protection Council, Monograph* 41: 420 pp. [proceedings].

1989 First Australasian Workshop on Pelagic Molluscs, Heron Island, Queensland, Australia, 8-11 October.

CIAC Newsletter 5(1):5-6, 1990 (September).

1989 Grand Forum de la Malacologie et Symposium International d'Écophysiologie des Mollusques, Ile de Embiez, Var, France, 16-21 October.

Haliotis 20: 206 pp., 1991 [as "1990"].

1989 International Symposium on Mussels [Seminario Internacional do Mexillon], A Toxa (O Grove), Galicia, Spain, 6-10 November.

B. L. Bayne, ed., 1991, The biology and cultivation of mussels, *Aquaculture* 94(2-3):121-278.

Cuadernos da Área de Ciencias Mariñas, Santiago de Compostelo 6:1-213, 1992.

1990 Molluscan Introductions and Transfers: Risk Considerations and Implications, held at 82nd Annual Meeting of the National Shellfisheries Association and Shellfisheries Institute of North America, Williamsburg, Virginia, U. S. A., 4-5 April.

J. T. Carlton and A. Rosenfeld, eds., 1992 (December), *Journal of Shellfish Research* 11(2):485-546.

1990 Les Mollusques Marins: Biologie et Aquaculture, Brest, France, 9 November.

Haliotis 22: 172 pp., 1992 [Actes de Colloques 14].

1991 International Workshop on the Marine Bivalvia of California, Moss Landing, California, U. S. A., 5-19 July, Paul H. Scott and Brian Morton, organizers.

The Veliger 37(1):1-92, 1994 (3 January).

1991 Summer Mortality in Mussels, Quebec City, Quebec, Canada, April.

Bulletin of the Aquaculture Association of Canada 91(2):5-65, 1991 (June).

1991 Workshop on *Semisulcospira* Studies, Kyoto, Japan, 19 October.

The Chiribotan 22(4):100-102 [report by S. Nishiwaki].

1992 Biology, Mariculture, and Economics of Giant Clams, organized by W. Fitt, *et al.*, Seventh International Coral Reef Symposium, University of Guam Marine Laboratory, Guam, 22-27 June.

Proceedings of the Seventh International Coral Reef Symposium 2:739-753, 1993 [papers and abstracts].

1992 International Congress on Molluscan Conservation, Glasgow, Scotland, United Kingdom, 10-12 September.

1992 Workshop on Larval Ecology and Evolution of Gastropods, Rome, Italy, 8 October; Marco Oilverio, organizer, Centro Italiano Studi Malacologici.

Notiziario C. I. S. M. A., *Bollettino del Centro Italiano di Studi Malacologici* 16:5-13, 31 October 1995 ["1994"] [one paper only].

1992 Symposium on Freshwater Mussel Conservation and Management, St. Louis, Missouri, U. S. A., 11-14 October.

1993 Workshop on Freshwater Bivalves of Pennsylvania, Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, U. S. A., 6-7 May.

Arthur Bogan, ed., 1993, ii + 80 pp. [handbook].

1994 International *Partula* Workshop, London Zoo, London, England, United Kingdom, 11-13 February, sponsored by *Partula* Propagation Group (PPG).

The Conchologists' Newsletter 131:431-434, December 1994 [report by Jane Reynolds].

1994 Integrated Management of Mollusc Pests in Lower Input Crop Systems, Lelystad, The Netherlands, 24-25 March.

1994 Workshop on Freshwater Mussels, Leesburg, West Virginia, U. S. A., May.

1994 International Pearl Conference, Honolulu, Hawaii, U. S. A., 14-19 May.

Journal of Shellfish Research 13(1):325-354, June, compiled and ed. by C. Richard Fassler [abstracts].

1994 Freshwater Mussel Conservation and Management, St. Louis, Missouri, U. S. A., 11-14 October, K. Welke, organizer.

1995 First International Conference on Computer Modelling of Seashells, Tweed Valley, New South Wales, Australia, 2-7 January, K. Kawaguchi, C. R. Illert, and R. Santilli, organizers.

1995 Mollusc Symposium, Sydney, New South Wales, Australia, 4-7 July, Australasia Marine

Science Association, organized by Malacological Society of Australia: (1) Molluscs, habitats and impacts, (2) Phylogeny and speciation of marine invertebrates.

1995 International Symposium on the Paleobiology and Evolution of the Bivalvia and 5th Canadian Palaeontology Conference, Drumheller, Alberta, Canada, sponsored by Royal Tyrell Museum of Paleontology, Alberta, Canada, Paul A. Johnston, organizer, 29 September - 2 October.

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63rd ANNUAL MEETING
THE AMERICAN MALACOLOGICAL UNION
SANTA BARBARA, CALIFORNIA
JUNE 22-27, 1997

The 1997 meeting of the American Malacological Union will be held at the Radisson Hotel on the beach in Santa Barbara, California, from Sunday, June 22, to Friday, June 27. The meeting will be held jointly with the 30th meeting of the Western Society of Malacologists.

Two major symposia are scheduled:

(1) Deep-Sea Mollusca, convened by Jerry Harasewych [Division of Mollusks, National Museum of Natural History, Washington, DC 20560; (202) 786-2073; FAX: (202) 357-2343; mnhiv006@sivm.si.edu], an overview of the fauna of the deep sea and what it tells us about the evolution of the Mollusca and its adaptation to the deep sea.

(2) Traditional vs. Phylogenetic Systematics, convened by Gary Rosenberg [Malacology, The Academy of Natural Sciences, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103-1195; (215) 299-1033; FAX: (215) 299-1170; rosenberg@say.acnatsci.org].

There will also be a special session on the cephalopods of the North Pacific chaired by Eric Hochberg [Invertebrate Zoology, Santa Barbara Museum of Natural History, 2559 Puesta del Sol Rd., Santa Barbara, CA 93019; (805) 682-4711, ext. 318; FAX: (805) 569-3170; inverts@sbmnh.rain.org].

There will be a reception the first evening on the beach across from the hotel, a visit to a winery in the Cachuma Valley, and a banquet at the Santa Barbara Museum. Optional field trips on the 27th include a tour of fossil formations of the Santa Barbara area led by Lindsey T. Groves [Malacology, Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, CA 90007; (213) 744-3376, or -3485; FAX: (213) 746-2999; groves@usc.edu], a cruise to the Channel Islands, and an in-depth tour of the Santa Barbara Museum of Natural History.

Those traveling from the East Coast or Midwest may want to arrive on the West Coast a day earlier or to stay two days later to get the lowest possible air fares. The Radisson Hotel will be $109 per room [double occupany; *i. e.* $55/person]; lower-cost options for students will be available. There are a number of nearby excellent restaurants in a variety of price classes.

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IN MEMORIAM

Harald A. Rehder
1907 - 1996
Honorary Life President
American Malacological Union

Emile A. Malek

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CONTRIBUTOR INFORMATION

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Hillis, D. M. 1989. Genetic consequences of partial self fertilization on population of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* 7(1):7-12.

Seed, R. 1980. Shell growth and form in the Bivalvia. *In: Skeletal Growth of Aquatic Organisms,* D. C. Rhoads and R. A. Lutz, eds. pp. 23-67. Plenum Press, New York.

Yonge, C. M. and T. E. Thompson. 1976. *Living Marine Molluscs.* William Collins Son and Co., Ltd., London. 288 pp.

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